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August 7-11, 1972
Fort Collins, Colorado

Sponsored by

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COLORADO STATE UNIVERSITY

And

U.S. ENVIRONMENTAL PROTECTION AGENCY
Office of Pesticide Programs

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Preface

These proceedings are made available to participants in the first annual short course "Environmental Chemicals: Human and Animal Health," which was organized and presented by the Institute of Rural Environmental Health, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, Colorado, in cooperation with the Office of Pesticide Programs, Environmental Protection Agency.

The course was developed to update current knowledge about the relationships and interactions of chemicals in the environment and their effects on human and animal health and to provide a forum for exploration of these relationships by various disciplines. Topics represented a wide range of thought, and we hope students will use the course proceedings to further stimulate their own knowledge of and concern for influences of chemicals on the environment.

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ENVIRONMENTAL GEOCHEMISTRY IN MISSOURI-- A MULTIDISCIPLINARY STUDY*

J. J. Connor, G. L. Feder, J. A. Erdman, and R. R. Tidball

Abstract

Current interest in the geochemistry of the natural environment as it may affect human and animal health prompted the U.S. Geological Survey to begin a wide-ranging program of geochemical studies in the State of Missouri. Out of this program has emerged a general 4-stage sampling plan suitable for geochemical surveys of large regions; the number and choice of stages executed in any one survey depend on the detail of information required. The great diversity of natural materials in Missouri includes at least eight broad geohydrologic units, some 150 named rock units, more than 275 named soil series, and six vegetation-type areas encompassing 2400 native plant species; and it poses a difficult challenge in sampling design. Work to date has centered on the search for and definition of a minimum number of geochemically coherent categories of environmental materials whose members will exhibit as much geochemical uniformity as possible in comparison with differences among categories. Preliminary data indicate that (1) the geohydrologic units are geochemically distinct, (2) the rocks consist of perhaps as few as six important broad-scale geochemical types, (3) the soils are chemically indistinct when classified by conventional criteria and are better characterized by landscape type, and (4) vegetation-type areas as measured by selected plant species and associated soils appear to be distinct in trace-element composition.

Scope and Purpose of Study

The recent increased concern about the role of trace elements in health and nutrition has spurred an interest in the distribution and availability of nearly all elements in the environment. Probably the best known hazardous elements in the environment are Pb and Hg, but medical researchers (3) suggest that Ag, Cd, Sn, Ti, Al, Bi, Au, Ga, Ge, and Zr may also be "environmental contaminants" in living tissue. The same researchers note that Cu, Zn, Mn, Co, Mo, Se, Cr, Fe, and I have been identified as trace elements essential to biochemical function of some kind and that Rb, B, Ba, Sr, F, V, Ni, As, and Br (as well as some of the previously mentioned elements) appear to be ubiquitous in plants and animals.

The interest of medical workers in the trace-element environment prompted the U.S. Geological Survey to start a geochemical survey of the natural environment in the State of Missouri. The survey was begun in July 1969 in cooperation with the Environmental Health Center of the University of Missouri. The major purpose of the survey is to provide useful background or baseline data on the general geochemical setting in the State, which will allow an accurate assessment of local geochemical conditions.

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*Reprinted from Geology and the Quality of Life, Symposium 1, International Geological Congress, 24th Session, Montreal, Canada, 1972.

The survey consists of four parts directed at the chemistry of the rocks, the waters, the soils, and the vegetation. The primary objective is to determine the regional variations in the chemical characteristics of each of these materials. This information will be used by epidemiologists of the Environmental Health Center in searches for relationships between geochemistry and human or animal health. It may also be useful in obtaining a better understanding of the mechanisms operating in the transfer of trace elements between media.

The great diversity of natural materials in Missouri includes eight broad geohydrologic units, about 150 named rock formations, more than 275 named soil series, and about 2400 native plant species that occur in six major vegetation-type areas. This diversity is a difficult challenge to effective environmental characterization and requires a carefully planned sampling program in order to obtain maximum information from minimum sampling and analytical costs.

Sample Design

The sampling designs in this program are based on broad categories within each of the major natural sampling media--rocks, waters, soils and plants. Each broad category is chosen such that its components will exhibit as much geochemical uniformity as possible in comparison with differences among categories. For example, rock categories are defined on a lithologic basis because geochemical variation within a lithologic type is expected to be small in comparison to the variation between types. After the sampling categories are defined, the sampling can proceed in as many as four stages:

Phase 1: Sampling to describe differences among categories.

Stage 1a: Preliminary sampling designed to determine the extent to which the categories are indeed geochemically distinct, and to provide the basis for planning Stage 1b.

Stage 1b: Final sampling to derive reliable estimates of differences among categories, and the amounts of compositional variability within each category.

Phase 2: Sampling to describe patterns of variation within categories.

Stage 2a: Preliminary sampling within each category to determine the sampling locality spacing that would be most efficient for describing the geochemical variation patterns within each category and the number of samples required from each locality.

Stage 2b: Final sampling to describe the geochemical patterns within each category.

The basic statistical model for Stage 1a sampling is based on the hierarchical case of the analysis of variance and is used to estimate components of geochemical variation thought to be important. Components commonly estimated are those reflecting (1) variation among categories, (2) variation at large sample intervals within categories, (3) variation at shorter sampling intervals, and (4) variation in laboratory procedures (analytical reproducibility).

This model is written:

$$X_{ijkl} = \mu + \alpha_i + \beta_{ij} + \gamma_{ijk} + \delta_{ijkl} \qquad (1)$$

where X_{ijkl} is the value of some element reported by the analyst and μ is the average concentration for that element in the medium under study. The remaining terms reflect the combined effects of the four components of variation in that particular analytical value. They are assumed to be random variables with means of zero and variances of σ_α^2, σ_β^2, σ_γ^2 and σ_δ^2, and are estimated by subdividing the total observed variance into four parts:)

$$s_X^2 = s_\alpha^2 + s_\beta^2 + s_\gamma^2 + s_\delta^2 \qquad (2)$$

where s_X^2 is the observed variance in X, and the four terms on the right are estimates of the four components of interest. Components may be added to or deleted from (1) or their definition may be changed to meet the peculiarities of a particular field problem. Because most trace-element data in environmental geochemistry approximate a log-normal frequency distribution, the variances are commonly derived from logarithmically transformed data.

It may happen that the results of Stage Ia sampling adequately distinguish among categories and that Stage Ib sampling is not necessary. A quantitative assessment of this contingency is possible by comparing the among-category variance (s_α^2) to the variance of the category means computed from the Stage Ia sampling results. The variance of the category means is estimated from:

$$s_m^2 = \frac{s_\delta^2}{n_\delta n_\gamma n_\beta} + \frac{s_\gamma^2}{n_\gamma n_\beta} + \frac{s_\beta^2}{n_\beta} \qquad (3)$$

where s_m^2 is the variance of a category mean and n_δ, n_γ, and n_β are the numbers of sampling units at each of the three lower levels of the design. The ratio:

$$V_m = s_\alpha^2/s_m^2 \qquad (4)$$

compares the magnitude of the category differences to the imprecision in estimating those differences. Preliminary computer simulation experiments suggest that the absolute minimum acceptable value of V_m is about 1.0. Where V_m is less than 1.0, additional sampling (Stage Ib) is required.

Environmental Categories

The rocks. The distribution of rock units in Missouri is shown in Figure 1. Overlying the bedrock units in northern Missouri are extensive deposits of glacial materials and in southern Missouri extensive deposits of clay-rich residuum resulting from the weathering of carbonate bedrock. The six most important categories of geologic materials in Missouri, in an environmental sense, are (1) glacial deposits of Quaternary age, (2) carbonate residuum of Cenozoic age, (3) shale and (4) limestone, both of Pennsylvanian age, (5) limestone of Mississippian age, and (6) dolomite of Cambrian-Ordovician age.

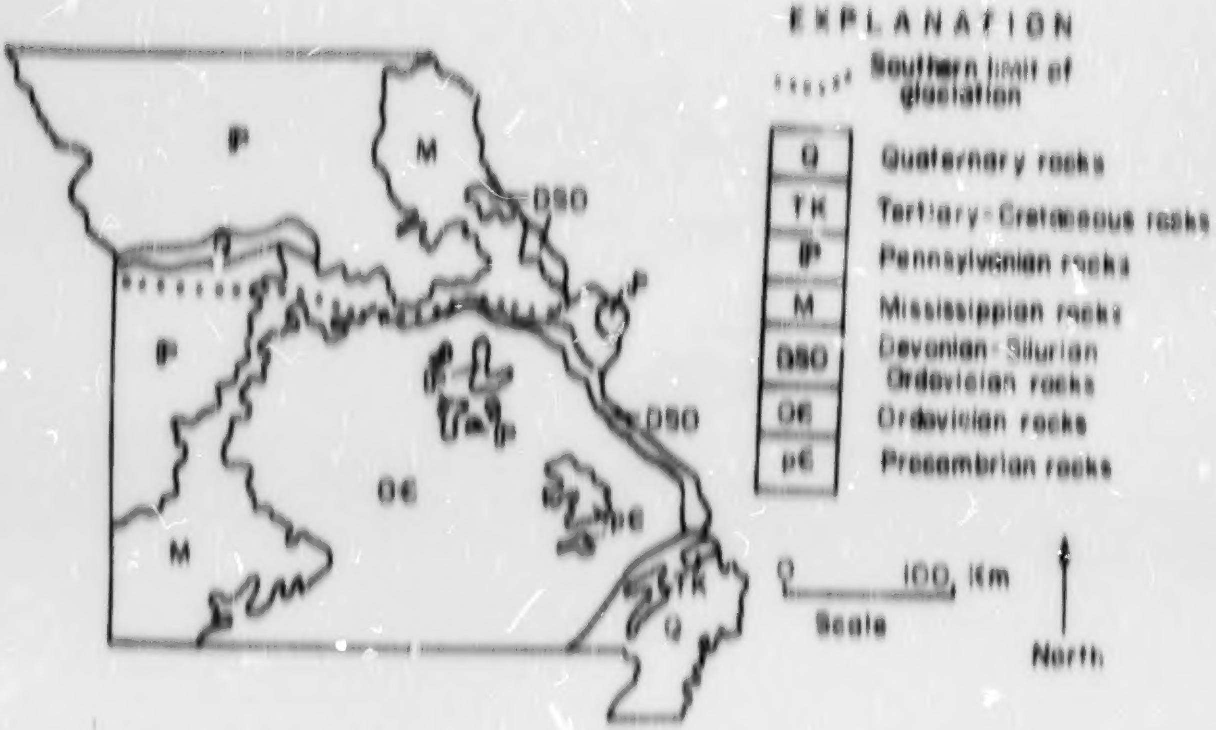

EXPLANATION
Southern limit of glaciation
Q Quaternary rocks
TK Tertiary-Cretaceous rocks
P Pennsylvanian rocks
M Mississippian rocks
DSO Devonian-Silurian Ordovician rocks
O€ Ordovician rocks
p€ Precambrian rocks
0 100 Km
Scale
North
P
M
DSO
P
O€
p€
M
TK
Q

Figure 1. Generalized geologic map of Missouri. Modified from American Association of Petroleum Geologists (1966).

The carbonate residuum, Category 3, consists of a red, clay-rich, cherty material representing the accumulated residue of subaerial weathering of carbonate bedrock. In general, the shale contains more trace elements in greater concentrations than rocks in the remaining five categories; loess, which is related to the glacial deposits of Category 2, tends to be high in the alkali and alkaline earth metals; and the limestones and dolomites are the poorest in trace elements.

The geochemical disparity among these categories is obvious and the among-category component of variance was not included in the statistical model used for rock sampling. Instead, separate models were defined for each category. Each model included a stratigraphic component of variance reflecting variation across the geologic section as well as geographic and analytical components.

The waters. Seven geohydrologic categories of potable ground water have been sampled in Missouri. An eighth category--ground water in the Precambrian crystalline rocks--is of such limited use that it was excluded from this study. The geohydrologic categories closely parallel some of the major rock categories because of the important physico-chemical control exercised by the rock reservoir on the contained water.

The geohydrologic units are (1) water from the Quaternary alluvium, (2) water from Quaternary glacial drift, (3) water from Cretaceous and Tertiary strata, (4) water from Pennsylvanian strata, (5) water from Mississippian strata, (6) water from Cambrian-Ordovician strata in southwestern Missouri, and (7) water from Cambrian-Ordovician strata in southern and southeastern Missouri.

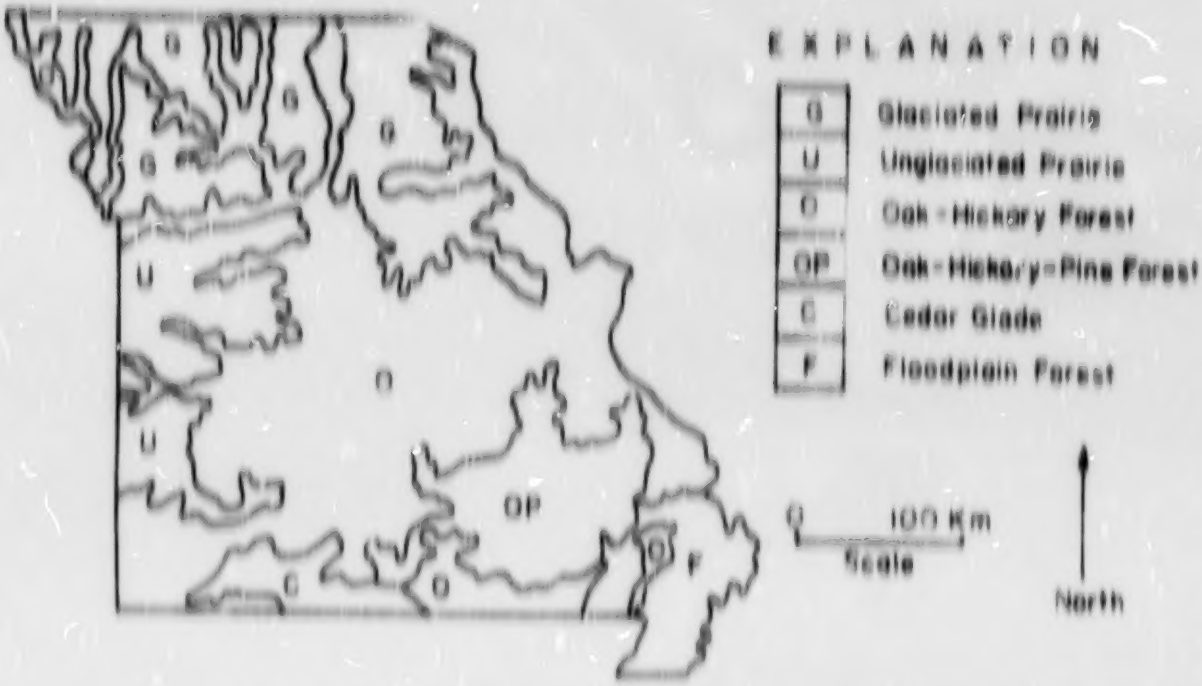
In general, ground water from the glacial drift and from the Pennsylvanian strata contains higher concentrations of trace elements than ground water from the other units.

The statistical model used in the Stage 1a sampling of ground waters contains four components, as given in Equation 1.

In addition to estimating the variance among the seven geohydrologic units (categories), large-scale variation within units was estimated by sampling water from five randomly selected townships within each unit; small-scale variation was estimated by sampling two wells within two of the five townships; and sample variation (including analytical reproducibility) was estimated by sampling one well in each unit twice at randomly selected intervals over a period of three months.

The soils. Category definition has proved to be less tractable among Missouri soils than for the other media. There is a great diversity in the soils as expressed in the taxonomic criteria of the standard soils classification system (4). This system contains six taxonomic levels and some 275 soil series (one of the lower taxonomic level) in Missouri. A preliminary study based on the A-horizon (or surface layer) was used to estimate the kind and magnitude of chemical variability associated with three of these taxonomic levels (5). The results demonstrate that the use of soil series as sampling categories would permit description of some 60% of the total compositional variability in Missouri soils, but such categorization would result in unacceptably large sampling and analytical costs. Therefore, attention has focused on alternative classification criteria.

Another study of the chemical variability of Missouri soils was based on variation in the B-horizon of uncultivated soils in the six vegetation-type areas of the State (Figure 2). The data obtained indicate that, for purposes of describing geochemical variations, classification of the soils by vegetation-type area is more effective than classification by the standard system. The vegetation-type areas in Missouri are (1) Floodplain Forest (soils here are developed largely on alluvium along the Mississippi River), (2) Glaciated Prairie (soils here are developed mostly on glacial deposits), (3) Unglaciated Prairie (soils here are developed mostly on shale and limestone), (4) Cedar Glade Forest (soils here are developed mostly on carbonate residuum), and (6) Oak-Hickory-Pine Forest (soils here are developed on granitic rocks, sandstone, and carbonate residuum).


G
G
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OP
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O
C
F
EXPLANATION
G Glaciated Prairie
U Unglaciated Prairie
O Oak-Hickory Forest
OP Oak-Hickory-Pine Forest
C Cedar Glade
F Floodplain Forest
0 100 Km
Scale
North

Figure 3. Generalized vegetation-type areas in Missouri. Modified from Kuchler (1964).

B-horizons of uncultivated soils from both prairie areas generally appear to be the richest in trace elements of any in the State, whereas B-horizons of uncultivated soils from the Oak-Hickory-Pine Forest are the poorest in trace elements.

The statistical model for Stage 1a and Stage 1b sampling in this study also contained four components of variation. One component reflected differences among soils from the six vegetation-type areas, a large-scale component reflected differences among soils from different 7½ minute quadrangles within each area (3 quadrangles were sampled in each vegetation-type area in Stage 1a; 10 in Stage 1b), and a small-scale component reflected differences among soils within quadrangles (2 samples were collected within quadrangles in Stage 1a; 3 in Stage 1b). A component of analytical reproducibility for these soil samples was estimated from an independent study for Stage 1a but was estimated as an integral part of the experiment in Stage 1b.

Additional studies of chemical variability among Missouri soils are directed at the importance of parent material in determining chemical characteristics and at the chemistry of the surface horizons of agricultural soils.

The plants. About 2400 distinct native plant species have been recognized in Missouri; because their complete chemical characterization is impossible, the main effort in the plant study is directed at the chemistry of two species that are widespread throughout the State. The dominant species in each vegetation-type area are also being studied (Figure 2).

The two selected widespread species are smooth sumac (*Rhus glabra*) and buckbrush (*Symphoricarpos orbiculatus*) and are intended to serve as indicator plants which may reflect geographic differences in plant response to the geochemical environment. Samples of smooth sumac were taken at each site where the B-horizon of uncultivated soils was sampled. The statistical model and sampling plan, therefore, are identical to those for uncultivated soils previously described. Samples of buckbrush were collected during Stage Ib sampling as an alternative indicator species.

Geochemical Variability in Missouri and its Implications

Estimates of some of the variance components derived from Phase I sampling of ground water, uncultivated soil, and smooth sumac over the entire State are shown in Table 1. Most elements in ground water exhibit geographic variance components, like those of Sr and carbonate hardness. That is, variation between wells within a township is small compared with variation on larger scales (between townships). Commonly, the variation among townships within geohydrologic units is as great or greater than that among units. However, pH is the only chemical property measured that yielded a ratio, V_m, much below unity. Most of the ratios are considerably higher, and the results of Stage Ia sampling are deemed sufficient for obtaining satisfactorily stable estimates of the average differences among the waters from the seven broad geohydrolic units in the State. It also seems likely that environmental responses to most aspects of ground-water chemistry, if present, should vary as much or more within the major parts of the State underlain by the geohydrologic units as they do among those parts. In general, they should not vary greatly within small areas.

The double entries for uncultivated soils in Table 1 are completely independent estimates of the variance components obtained from Stage Ia and Stage Ib sampling. Most elements in the soils have variance components similar to those of Cu and V. That is, variance between quadrangles widely distributed over the vegetation-type areas is small in comparison with that between areas and between sampling sites within quadrangles. This property of the variance was exploited in the Stage Ib sampling by increasing the sampling density within quadrangles and sampling only 10 quadrangles. Thus, although five times as many samples were collected in Stage Ib as in Stage Ia, it was necessary to sample only twice as many quadrangles. As shown in Table 1, the results of the Stage Ib sampling yielded substantially higher V_m ratios, and, hence, substantially more stable estimates of the chemical differences among the soils of the six vegetation-type areas. As for the possible epidemiological implications, any plant and animal responses to the chemistry of the soils in Missouri can reasonably be

TABLE 1

Components of logarithmic variance of selected geochemical properties in ground water, uncultivated soil, and stems of smooth sumac in Missouri.

Ground Water

Variable	Between Samples (s_δ^2)	Between Wells (s_γ^2)	Between Townships (s_β^2)	Between Geohydrologic Units (s_α^2)	Variance of Unit Means (s_m^2)	(V_m)
pH[1]	0.0250	0.0125	0.1516	0.0070	0.0378	0.19
Li	.0090	.1793	.1639	.3421	.0764	4.48
Sr	.0018	.0150	.1402	.1370	.0314	5.00
Carbonate hardness	.0004	.0081	.0954	.0479	.0208	2.31

Uncultivated Soils (A-horizon)
(Upper entry estimated from results of Stage Ia sampling;
lower entry from Stage Ib sampling)

Variable	Between Analyses (s_δ^2)	Between Sites (s_γ^2)	Between Quadrangles (s_β^2)	Between Vegetation-Type Areas (s_α^2)	Variance of Unit Means (s_m^2)	(V_m)
Ba	0.0056 .0131	0.0292 .0187	0.0063 .0163	0.0177 .0212	0.0047 .0023	3.73 9.22
Cu	.0116 .0349	.0171 .0285	.0270 .0090	.0085 .0091	.0083 .0022	1.03 4.20
Sc	.0023 .0084	.0208 .0168	.0030 .0065	.0314 .0206	.0029 .0012	10.83 17.85
V	.0060 .0052	.0275 .0264	.0062 .0074	.0345 .0287	.0113 .0014	3.05 20.92

Smooth Sumac (Stems)

Variable	Between Analyses	Between Sites ($s_\delta^2+s_\gamma^2$)	Between Quadrangles (s_β^2)	Between Vegetation-Type Areas (s_α^2)	Variance of Unit Means (s_m^2)	(V_m)
Mn	No data	0.0240	0.0270	0.0203	0.0078	2.60
B	No data	.0133	0	0	.0013	0
Ba	No data	.0779	.0289	.1412	.0136	10.38
Cu	No data	.0171	.0061	.0097	.0029	3.75
Pb	No data	.0482	.0013	.0079	.0051	1.55

[1] Components of variance in pH were computed in terms of standard units.

expected to vary among the major vegetation-type areas. Also, little variation in response should be expected on a broad scale within vegetation-type areas--assuming, of course, that the response is a direct and simple effect of some element, or combination of elements, having the type of variability that has been found for most elements. Where the response results from a combination of environmental factors, of which soil chemistry is only one, its pattern of variation will be less predictable.

Stems of smooth sumac, like the B-horizons of uncultivated soils, vary comparatively little in composition over broad parts of the same vegetation-type area, but do tend both to vary among areas and to vary locally within areas (between sites). However, there appears to be little correspondence between element concentrations in sumac stems and element concentrations in the soil. Comparison of mean element concentrations in the two media in each vegetation-type area indicates that of those elements studied, only Fe in sumac apparently reflects the elemental soil concentrations. Element concentrations in sumac, therefore, may be related more to soil pH and soil moisture, which affect availability, than to the total element concentrations in the substrate.

In conclusion, the sampling approach being tested in the Missouri survey appears to be practical for problems arising in geochemical surveys of large regions. Phase 1 sampling has identified the gross geochemical variations over the State and can provide epidemiologists with what may be regarded as first-order geochemical maps--that is, maps showing the chemical differences in the rocks, soils, plants, and water among the major parts of the State. If more detailed information on the geochemical variability is needed, Phase 2 sampling may be done in order to describe the geochemical variation patterns within parts of the State. Whether or not more detailed information is needed will not be known until the data from Phase 1 have been thoroughly examined by the epidemiologists.

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TRACE ELEMENTS IN WATER

Albert V. Soukup

"Good afternoon, ladies and gentlemen, this is your local radio station, K- - -, with its regular 'phone-in-and-talk program. Just call 123-4567 and you will be on the air." Buzz, buzz. "This is Joe Crackpot. I've just put cyanide into the city water supply!"

Sound far fetched? No, not indeed! Essentially, this event occurred within the last three months in a city in this part of our nation. Luckily, it was only a false alarm.

How does this relate to trace elements in water? Perhaps we need to know the significance of these elements and their effect on Homo sapiens and/or animals. Our Congress is interested, as stated in a proposed amendment, #410, to Senate Bill 1478, which states

> ....that increasing quantities and types of pesticides, organic chemicals, toxic metals, and other contaminants are entering the public water systems that serve as sources which supply the nation with water for drinking, recreation, and other human uses; that many of these new contaminants are either not detected or not removed by established water testing and treatment methods; and these contaminants are consumed by the public or otherwise come into contact with the public thereby presenting a potential hazard to the public health.

They have proposed in the bill that research be done on

> (A) improved methods and procedures to identify and measure the existence of pesticides, toxic metals, organic chemicals, radioactive substances, bacteria, viruses, and other contaminants in water used for drinking, recreation, and other human uses;
>
> (B) improved methods and procedures to identify and measure the health effects of pesticides, toxic metals, organic chemicals, radioactive substances, bacteria, viruses, and other contaminants in water used for drinking, recreation, and other human uses."

Hundreds of new chemical compounds are being introduced into our environment annually. Few of these are assessed for their potential impact on the health of man, particularly on the synergistic effect they may have when acting together or in concert with other types of environmental stresses. Some of these chemicals, although at higher concentrations than are likely to be found in water, have been shown to cause cancer, genetic damage, or malformations at birth.

The impact of the long-term ingestion of low levels of chemical contaminants is very difficult to ascertain. Because the effects of such chemicals ingested in low concentrations over long periods are slow and insidious, and are likely to be similar to those effects manifested by aging or other chronic diseases, their significance is particularly difficult to establish.

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However, we know that carcinogens, mutagens, and teratogens are being discharged in waste waters and become part of the water supply of a substantial portion of the population. For example, waste waters in England have been shown to contain two well-established carcinogens; 3,4-benzpyrene and 1,2-benzanthracene, albeit at low concentrations.

Other hazards likely to be found in polluted waters include petroleum products and aromatic amino and nitro compounds. Radioisotopes, particularly in concert with other chemicals, constitute cancer hazards to the general population.

The highly toxic pesticides are particularly troublesome, often not because of the toxic effect of the chemical itself, but because of highly toxic impurities that result from their manufacture.

Monitoring of these chemicals and tracing their fate in our water management programs and treatment processes is absolutely essential if we are to assure Americans of the safety of their public water supplies. We must not learn of the health effects of these chemicals after they have occurred, thereby using Americans as guinea pigs.

Now, to the esthetic quality of water. Rapidly growing population and increasing rates of urbanization and industrialization are exerting greater and greater pressures on our limited water resources.

Although nature renews the volume of fresh water available, we have been profligate in its use so that communities have had increasingly to turn to polluted sources to meet their needs. Where once we boasted of the salubriousness and the quality of our water supplies, now we grudgingly accept the product provided, often with distaste. We have all seen the signs reading somewhat as follows: "Please flush the toilet, our neighbors downstream need the water."

Waters drawn from highly contaminated sources require the addition of many chemicals that may render the water distasteful, and many people have turned to bottled waters. Ironically, bottled waters do not undergo the surveillance that public supplies do and are often less safe, as shown recently by the Washington Star.

Unless we make a commitment to improve public water supply systems, our proud heritage of quality water will havr been lost.

It is argued sometimes that trace elements of unknown relationship should be tolerated in waters because no immediate relationship to man is apparent. Conversely, we should not wait for the best criteria, as stated by Dr. Abel Wolman. To paraphrase him, the best criterion for detecting a dangerous public water supply is the doctor's certificate showing that the man is dead and the epidemiologic evidence showing that the water he drank killed him.

So then, how do we consider limits of trace elements? These considerations must include the element or compound, its effect upon health and well-being, the allowable limit, and the concentration in the water versus the

Table 1

Health and aesthetic significance of trace elements and compounds and their occurrence in U.S. water supplies.

Trace Element or Compound	Health or Aesthetic Effect	1962 USPHS Drinking Water Standards Limit mg/l	Occurrence in U.S. Water Supplies 2,595 Distribution Samples (1969) mg/l	
			Average	Range
Alkyl benzene sulfonate (ABS)	USPHS limit gives a safety factor of 15,000; not highly toxic.	0.5*	0.05	0-0.41
Arsenic (As)	Serious systemic poison--100 mg usually causes severe poisoning, is cumulative, and may cause chronic effects. Late evidence indicates tiny amounts may be beneficial.	0.01* 0.05†	0.0001	<0.01-0.10
Chloride (Cl^-)	Limit set for taste reasons.	250*	27.6	<1.0-1,950
Copper (Cu)	Body needs copper at level of about 2 mg/day for adults; not a health hazard except when large amounts are ingested.	1*	0.13	0-8.35
Carbon Chloroform Extract (CCE)	At limit stated, organics in water are not considered a health hazard.	0.2*	0.11	0.008-0.56
Cyanide (CN^-)	Rapid fatal poison, but limit set provides safety factor of about 100.	0.01* 0.2†	0.00009	<0.1-0.008
Fluoride (F^-)	Beneficial in small amounts; above 2,250 mg dose can cause death.	0.7-1.2* 1.4-2.4†	0.33	<0.2-4.40
Nitrate (NO_3)	Excessive amounts can cause methemoglobinemia (blue baby) in infants.	45*	6.3	<0.1-127
Sulfate (SO_4)	Above 750 mg/l usually has laxative effects.	250*	46	<1.0-770
Zinc (Zn)	Zinc is beneficial in that a child needs 0.3 mg/kg/day; 675-2,280 mg/l may be an emetic.	5*	0.19	0-13.0
Barium (Ba)	Fatal dose is 550-600 mg as the chloride; muscle (including heart) stimulant.	1.0†	0.034	0-1.55
Cadmium (Cd)	13-15 ppm in food has caused illness.	0.01†	0.003	<0.2-3.94
Chromium (Cr^{+6})	Limit provides a safety factor; Carcinogenic when inhaled.	0.05†	0.0023	0-0.079
Lead (Pb)	Serious, cumulative body poison.	0.05†	0.013	0-0.64
Selenium (Se)	Toxic to both humans and animals in large amounts. Late research suggests small amounts may be beneficial.	0.01†	0.003	0-0.07
Silver (Ag)	Can produce irreversible, adverse cosmetic changes.	0.05†	0.008	0-0.03
Radium (Ra-226)	A bone-seeking, internal alpha emitter that can destroy bone marrow.	3 pc/l‡	2.2 pc/l	0-135.9 pc/l
Strontium (SR-90)	A bone-seeking, internal beta emitter.	10 pc/l‡	<1.0 pc/l	0-2.0 pc/l

* Not to be exceeded where other more suitable supplies are, or can be made, available.
† Excess constitutes grounds for rejection of the supply.
‡ Consult 1962 "USPHS Drinking Water Standards" for interpretation.

allowable limit. Table 1 indicates limits of the U.S. Public Health Service *Drinking Water Standards* (1), and Table 2 indicates limits not found in that manual but generally followed by the Water Supply Division of the Environmental Protection Agency. These two tables are reproduced through the courtesy of Floyd B. Taylor, Sanitary Engineer Director of the U.S. Public Health Service, from a paper presented at a Pacific Northwest Section meeting of the American Water Works Association on May 13, 1971. Table 3 indicates surface water criteria for public water supplies (6).

Conventional water treatment as commonly used in this country includes coagulation using alum, ferric sulfate, or other chemicals as necessary, but without coagulant aid or activated carbon, sedimentation (6 hours or less), rapid sand filtration, and disinfection with chlorine without consideration to concentration or form of chlorine residual.

Table 4 shows the current status of treatment capabilities for all the substances listed in the 1962 Public Health Service *Drinking Water Standards* (1).

Conventional water treatment processes are not effective in the removal of many organics (as represented in the carbon chloroform extract), chlorides, flourides, nitrates, odor, phenols, sulfates, or total dissolved solids. To some extent, the concentrations of some heavy metals such as lead, arsenic, cadmium and mercury are reduced during conventional treatment, but the effectiveness of the processes is unpredictable and therefore unreliable.

In those plants where water softening by chemical precipitation is practiced, the removal of heavy metals is enhanced but often incomplete. Activated carbon will remove most of the organics and odor. Other special treatment processes are available for the removal of arsenic, fluoride, iron, and manganese. At present, no reliable processes adaptable to large water treatment plants are available or known for the removal of barium, cadmium, chromium, lead, selenium, or mercury.

Serious health problems can arise when potential chemical pollutants in the environment find their way into the drinking water. It is important to keep in mind that poisoning by any of these substances may be not only acute, but also chronic. One of the most insidious and difficult-to-measure chronic effects is the production of cancers.

The metalloid arsenic may find its way into drinking water by various means, including deliberate placement. Contamination of streams with arsenical wastes can lead to a percolation of this renowned poison into ground waters used as a source of drinking water, or water from the contaminated stream may be pumped directly into a municipal supply. In some areas, arsenic in the water comes from geologic formations. Ordinary filtration techniques usually are not efficient in the removal of arsenic; consequently, special means must be employed.

The toxicity of arsenic is well known; the ingestion of as little as 100 milligrams usually results in severe poisoning. Chronic arsenic poisoning

Table 5

Health and aesthetic significance of trace elements and compounds in finished drinking water: elements not found in 1962 "USPHS Drinking Water Standards"

Name of Ingredient	Health or Aesthetic Effect	Unofficial Limit mg/l	Occurrence in U.S. Water Supplies mg/l	
			Average	Range
Substance				
Antimony (Sb)	Similar to arsenic but less acute. Recommended limit [illegible] to exceed 0.1 mg/l; [illegible] below 0.05 mg/l; over long-time periods [illegible] 0.01 mg/l.	0.05		
Beryllium (Be)	Poisonous in some of its salts in occupational exposure.	None		
Bismuth (Bi)	A heavy metal in the arsenic family--avoid in water supplies.	None		
Boron (B)	Ingestion of large amounts can affect central nervous system, and protracted ingestion can cause borism.	1.0	0.069	0-3.28*
Cobalt (Co)	Beneficial in small amounts; about 7 μg/day.	None	0.002	0.0-0.019*
Molybdenum (Mo)	Necessary for plants and ruminants. Excessive intakes may be toxic to higher animals; acute or chronic effects not well known.	None		
Mercury (Hg)	Continued ingestion of large amounts can damage brain and central nervous system.	0.005	0.000	0.000-0.013†
Nickel (Ni)	May cause dermatitis in sensitive people; doses of 30-73 mg of [illegible] have produced toxic effects.	None	0.003	0.0-0.072*
Sodium (Na)	A beneficial and needed body element, but can be harmful to people with certain diseases.	20‡	40% above 20 mg/l	0.4-1,900§
Tin (Sn)	Long used in food containers without known harmful effects.	None		
Uranyl ion (UO_2)	May cause damage to kidneys.	5.0		
Vanadium (V)	Some evidence that vanadium may be beneficial with respect to heart disease.	None		
Pesticides				
Aldrin	One or all of these complex organic compounds have severe, acute, adverse health effects when ingested in large amounts. Small amounts accumulate, and long-time effects are generally unknown.	0.017**	180 samples in NCWSS showed lower values than the limits.	
Chlordane		0.003**		
DDT		0.042**		
Dieldrin		0.017**		
Endrin		0.001**		
Heptachlor		0.018**		
Heptachlor epoxide		0.018**		
Lindane		0.056**		
Methoxychlor		0.035**		
Toxaphene		0.005**		
Organic phosphorous plus carbamates, such as Parathion, Malathion, Carbaryl, and others	The organic phosphorous and carbamate pesticides are severe acute poisons affecting the central nervous system. Ingestion of small amounts over time can harm the central nervous system.	0.1††	Same as for pesticides.	
Herbicides				
[illegible]	A group having toxic properties of a generally lower order than the above pesticides. However, they should not be used without great care and should not be found in drinking water.	1.0		
[illegible]		1.0 [illegible]		
[illegible]		1.0 [illegible]		
[illegible]		1.0 [illegible]		
[illegible]		1.0		
[illegible]		0.1		
2,4-D		0.1		
2,4,5-T should not be used.		1.0		
[illegible]				
[illegible]		0		
[illegible]		1.0		
[illegible]		0.1		
[illegible]		0.1		
[illegible]		[illegible]		
[illegible]		0.1		
[illegible]		1.0 [illegible]		

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* National Community Water Supply Study: 2500 samples.
† [illegible]
‡ The amount of, and above, which people on strict (500 mg) sodium-restricted diets must include in their daily sodium intake calculations.
§ Survey of over 2,000 U.S. municipalities by USPHS in 1963-66.
** Report of the Committee on Water Quality Criteria, FWPCA, U.S. Department of the Interior.
†† As parathion equivalent in cholinesterase inhibition.

Table 3

Surface water criteria for public water supplies.

Constituent or Characteristic	Permissible Criteria	Desirable Criteria	Paragraph
Physical:			
Color (color units)	75	<10	1
Odor	Narrative	Virtually absent	2
Temperature*	do	Narrative	3
Turbidity	do	Virtually absent	4
Microbiological:			
Coliform organisms	10,000/100 ml†	<100/100 ml†	5
Fecal coliforms	2,000/100 ml†	<20/100 ml†	5
Inorganic chemicals:			
	(mg/l)	(mg/l)	
Alkalinity	Narrative	Narrative	6
Ammonia	0.5 (as N)	<0.01	7
Arsenic*	0.05	Absent	8
Barium*	1.0	do	8
Boron*	1.0	do	8
Cadmium*	0.01	do	8
Chloride*	250	<25	8
Chromium*, hexavalent	0.05	Absent	8
Copper*	1.0	Virtually absent	8
Dissolved oxygen	≥4 (monthly mean) ≥3 (individual sample)	Near saturation	10
Fluoride*	Narrative	Narrative	11
Hardness*	do	do	12
Iron (filterable)	0.3	Virtually absent	8
Lead*	0.05	Absent	8
Manganese* (filterable)	0.05	do	8
Nitrates plus nitrites*	10 (as N)	Virtually absent	13
pH (range)	6.0-8.5	Narrative	14
Phosphorus*	Narrative	do	15
Selenium*	0.01	Absent	8
Silver*	0.05	do	8
Sulfate*	250	<50	8
Total dissolved solids* (filterable residue)	500	<200	16
Uranyl ion*	5	Absent	17
Zinc*	5	Virtually absent	8
Organic chemicals:			
Carbon chloroform extract* (CCE)	0.15	<0.04	18
Cyanide*	0.20	Absent	8
Methylene blue active substances*	0.5	Virtually absent	19
Oil and grease*	Virtually absent	Absent	20
Pesticides:			
Aldrin*	0.017	do	21
Chlordane*	0.003	do	21
DDT*	0.042	do	21
Dieldrin*	0.017	do	21
Endrin*	0.001	do	21
Heptachlor*	0.018	do	21
Heptachlor epoxide*	0.018	do	21
Lindane*	0.056	do	21
Methoxychlor*	0.035	do	21
Organic phosphates plus carbamates*	0.1‡	do	21
Toxaphene*	0.005	do	8
Herbicides: 2,4-D plus 2,4,5-T plus 2,4,5-TP*	0.1	do	21
Phenols*	0.001	do	8
Radioactivity:			
	(pc/l)	(pc/l)	
Gross beta*	1,000	<100	8
Radium-226*	3	<1	8
Strontium-90*	10	<2	8

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* The defined treatment process has little effect on this constituent.

† Microbiological limits are monthly arithmetic averages based upon an adequate number of samples. Total coliform limit may be relaxed if fecal coliform concentration does not exceed the specified limit.

‡ As parathion in cholinesterase inhibition. It may be necessary to resort to even lower concentrations for some compounds or mixtures.

Table 4

Treatment capabilities for trace elements and compounds.

Substance	Controlled by conventional treatment	Essentially no removal by conventional treatment	Special treatment available
Alkyl benzene sulfonate		X	
Arsenic			X
Barium[1]			
Cadmium[1]			
Carbon chloroform extract		X	X
Chloride		X	
Coliform organisms	X		
Chromium (hexavalent)[1]			
Color	X		
Copper[1]			
Cyanide[1]			
Fluoride		X	X
Iron			X
Lead[1]			
Manganese			X
Nitrate		X	X
Odor		X	X
Phenol		X	X
Radioactivity			X
Selenium[1]			
Silver[1]			
Sulfate		X	
Total dissolved solids		X	
Turbidity	X		
Zinc[1]			
Mercury[1]			

[1] These elements are removed by conventional treatment to varying degrees. Unfortunately the degree of removal for different quality waters is unpredictable.

Coagulation, followed by settling and filtration is effective in removing turbidity, color, and to some degree bacteria, since many of these microorganisms are attached to the particles of turbidity. Coagulation and filtration, in removing suspended particles from the water, also render the water more responsive to disinfection agents.

is insidious in onset. The first symptoms are those that may attend many disorders. They consist of weakness, languor, anorexia, nausea, vomiting, and diarrhea or constipation. As poisoning progresses, symptoms become more characteristic, such as conjunctival congestion and catarrhal inflammation of the upper respiratory tract. Stomatitis, salivation, dermatitis, and hyperkeratosis may be observed. Liver involvement may lead to jaundice and eventually to cirrhosis. The kidneys are also damaged. Evidence of an effect on the capillary beds is indicated by the edema that occurs. The advanced stages of chronic intoxication are encephalopathy and peripheral neuritis. Severe blood dyscrasias are observed also in chronic arsenic poisoning.

The U.S. Public Health Service *Drinking Water Standards* of 1962 set a limit of 0.01 milligram of arsenic per liter in drinking water, with 0.05 milligram per liter as a rejection limit if other sources cannot be found.

For some time, the heavy metals have been a source of concern from a public health standpoint. Lead exemplifies this problem. Lead taken into the body can be seriously injurious to health, even lethal, if taken for either brief or prolonged periods. Prolonged exposure to relatively small quantities may result in serious illness or death. Lead is a cumulative poison and is dangerous when taken into the body even in relatively small quantities.

Three fairly distinct types of chronic lead poisoning are described: 1) the gastrointestinal, 2) the neuromuscular, and 3) the central nervous system (CNS) syndromes. They may occur separately or in combination. The neuromuscular and CNS syndromes tend to result more from intense exposure to lead, whereas the abdominal syndrome tends to develop from a very slow and insidious intoxication. The CNS syndrome is seen typically in children and is manifested by encephalopathy, visual disturbances, and eventually brain damage. Also, lead interferes with the normal permeability of the red blood corpuscles. Neuromuscular involvement is characterized by muscle weakness and fatigue; whereas, the gastrointestinal symptoms are nausea, vomiting, constipation, and a persistent metallic taste.

It is not uncommon to find the lead content of water in urban supplies to be in the microgram range of concentration, provided the water is not stored in tanks painted with oil-base lead paint and provided the pipes are not of lead or lead alloy. Principal sources of lead in drinking waters are lead pipes and goosenecks in houses and plumbing systems. The use of lead pipe is permitted to this date by many plumbing codes. The sources of lead are varied. In a case in New Orleans in 1959, ice used for mixing alcoholic beverages was kept in a lead-bottom container; one serious case of chronic lead poisoning was diagnosed. In New England, in years past, lead pipes were used to bring acid spring water to farms, and cases of lead poisoning resulted.

A lead concentration of 0.05 milligram per liter is the limit set by the U.S. Public Health Service Drinking Water Standards of 1962.

Cadmium is recognized as an element of high toxic potential. This metal is distributed widely in nature and is often used in metallurgy. Cadmium salts sometimes are used for insecticides and antihelminthics.

Cadmium poisoning of humans has resulted from the consumption of foods and liquids prepared and left in cadmium-plated containers. As a result, several health departments have forbidden the use of cadmium-plated food containers and pipes. Cadmium-contaminated ice cubes in cold drinks have caused acute gastritis symptoms within one hour; consumption of cadmium salts causes cramps, nausea, vomiting, and diarrhea. Oral ingestion of cadmium has been reported as the cause of a number of human deaths.

Cadmium tends to concentrate in the liver, kidneys, pancreas, and thyroid of humans and animals. Once it enters the body, it is likely to remain. Normally, many plant and animal tissues contain about 1 milligram of cadmium per kilogram of tissue, but there is no evidence that cadmium is biologically essential or beneficial.

Humans have become ill from ingestion of cadmium in concentrations of 13 to 15 milligrams per kilogram in popsicles, 67 milligrams per liter in a cold drink, and 530 milligrams per kilogram in gelatin. As little as 14.5 milligrams of cadmium taken orally has caused nausea and vomiting; yet, in another instance, as much as 330 milligrams has not caused permanent injury. Four members of a family whose drinking water contained 0.047 milligram of cadmium per liter had no history of adverse effects. The 1962 Drinking Water Standards of the U.S. Public Health Service set a mandatory maximum limit for cadmium of 0.01 milligram per liter.

Of the many organic water pollutants, dyes and their breakdown products make up a substantial part. Those dyes that are hydrophilic, non-polar, and most resistant to breakdown are most likely to reach the drinking water of various rural and urban communities. The type and concentration of dyes found in any particular waterway will depend primarily upon the types of industries along its banks. Most of the dyes in streams are industrial waste products; some, however, have been placed there for the purpose of studying the streams.

Several of the dyestuffs identified have been shown to be carcinogenic; consequently, they create a public health problem. Many times the dyes have a built-in safety factor because in high concentrations, they turn the water a characteristic color visible to the naked eye. At low concentrations and under conditions of chronic exposure, these substances may constitute a threat to the water consumer. Other than data concerning the carcinogenic property of the substances, relatively little chronic toxicity data are available. Webb and his co-workers (7) demonstrated that the chronic oral administration of Rhodamine B (a dye used in water-flow studies) impaired growth and affected the liver of rats. Dyestuffs, such as diaminoazobenzene and Bismark Brown (both industrial waste products) are highly toxic materials; in rural or municipal drinking waters they can cause many adverse reactions in the population. To date, no official limit has been set for the concentration of these chemicals in drinking water.

Pesticides are high on the list of chemicals beneficial to mankind. Their contributions to the productivity, improved comfort, and better health of the people are matched only by their toxic potential. Pesticides are categorized generally into three chemical groups: 1) the inorganics, comprising the arsenicals, mercurials, and fluorides; 2) the synthetic organics consisting of the chlorinated hydrocarbons, organic phosphates, and thiocarbamates, and 3) the natural organics, of which rotenone, pyrethrum, and nicotine are examples.

Pesticides may gain access to ground and surface waters through direct application, through percolation and runoff from treated areas, and/or through drift during application. Pesticides have entered the drinking water system accidentally when a reverse-pressure system existed. For example, a worker placed a water hose in a tank partially filled with chlordane concentrate without realizing that the water had been turned off at the main valve. The result was a siphoning of the pesticide into the distribution system. Gross over-application of pesticides is a common practice that augments the pollutional effects of pesticides to a considerable extent. These substances are entering our natural waters in ever increasing amounts.

When considering pesticidal pollution of water, toxicity is not the only concern. Difficult problems are posed also by tastes and odors. Some synthetic organics cause highly objectionable tastes and odors, as do many of their solvents.

The addition of synergists and/or adjuvants to make a particular pesticide more effective has been practiced by the pesticide manufacturing industry for many years. A marked synergistic effect, or potentiation, of up to fiftyfold in acute toxicity exists when some chemicals are administered simultaneously. Such effects cause an increased hazard to the health of individuals exposed to such preparations.

Each pesticide produces its own pattern of symptoms. Those most studied are the symptoms associated with organic phosphates and with chlorinated hydrocarbons. All of the symptoms observed with organo-phosphorus poisoning are referable to the inhibition of the enzyme acetylcholinesterase and the consequent continuous action of acetylcholine on the receptor neurons. Involuntary salivation, lacrimation, urination, and defecation along with muscle fasciculation are characteristic of poisoning by organic phosphorus insecticides. In contrast, the symptoms of poisoning produced by chlorinated hydrocarbons are loss of appetite and weight, emaciation, hyperirritability, tremors, convulsions, and coma; death, if it occurs, is by respiratory failure. Because these effects occur when relatively low concentrations are involved, and because there may be potentiation of toxicity, these chemicals are considered very hazardous, and all precautions should be taken to ensure that toxic concentrations are not introduced into the drinking water.

In conclusion, Dr. Henry Bergen of the University of Massachusetts has recently reiterated some questions which should be of immediate concern to us all. These are:

1. What are the long-term toxic and carcinogenic implications

of repeated exposure through drinking water to organics of industrial origin?

2. What are the toxic implications for the long term of trace metals occurring in drinking water, and how may these be controlled?
3. How can water treatment plants reliably destroy viruses in sources of supply?
4. Are the Public Health Service drinking water standards applicable to waters derived in large part from treated sewage?
5. What are the public health implications of deterioration of distribution systems at a time when these are being subjected to increasing use?

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MERCURY AS AN ENVIRONMENTAL POLLUTANT

Michael G. Petit

Introduction

There has been growing concern in recent years about toxic levels of environmental chemicals observed in natural substrates. In many instances, the element mercury is implicated as a pollutant (5) and the source can be traced to man's industrial or agricultural activity in the area. These instances represent examples of local pollution by mercury and are relatively easy to control once the sources are located. Of even greater concern is the problem of locating those sources of environmental mercury from which the effluent mercury has a more generalized distribution. These sources are more difficult to define because mercury from this source appears as a background level in substrates of broad general geographic distribution. This raises the important question: Are there any controllable sources which discharge mercury into a situation capable of providing a generalized geographical distribution? The answer to this question depends upon two factors: the nature of the effluent and the transport systems available for distribution.

Early work by Murozumi et. al. (3) using stratified annual ice sheets near Camp Century, Greenland, demonstrated that lead was a pollutant of global distribution. More recently, Weiss et. al. (6) extended the analysis of the ice sheets to include mercury. Their analytical results strongly suggest that mercury pollution is also of global distribution. These workers examined all known man-made sources of atmospheric mercury and found that the sum total of man's contribution was only a fraction of the total annual atmospheric burden. They suggested that natural degassing of the earth's crust and upper mantle was the major source of atmospheric mercury and that activities of man resulting in alteration of terrestrial surfaces must be included as a passive source of atmospheric mercury.

Environmental Substrates

If this is true, such geological processes as glacial movements followed by recession of the ice sheets should have resulted in the release of tremendous quantities of mercury into the atmosphere and, indeed, may still be. To examine this point further, it would be necessary to have access to an environmental substrate which was deposited during the Pleistocene and for which there exists a continuous or semicontinuous record extending into modern times. Although the chances of finding such a substrate which has remained essentially constant in composition since deposition are remote, guano deposits of the Mexican free-tailed bat *Tadarida brasiliensis* represent a close approximation.

Deposits of this material occur in dry solution type caves in the American Southwest and in volcanic tubes in tropical and temperate zones of worldwide distribution. Archeological sites such as the Anasazi ruins in Canyon de Chelly National Monument which are located in rock shelters and, as a result, are not exposed to weathering provide sources of material such as woodrat scat (*Neotoma spp.*) which can be accurately

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dated. Although this type of substrate is relatively modern, it was deposited well before the industrial revolution. Thus, these deposits provide us with a substrate which is suitable for determining baselines for environmental chemicals. This work reports results obtained from a dry cave (Eagle Creek Cave (2), 33°01' N lat, 109°25' W long) housing a large colony of the Mexican free-tailed bat, Tadarida brasiliensis.

Experimental

Three 6" diameter core samples were extracted from the guano deposits on the floor of the cave directly underneath the large vertical crack in the ceiling which extended up fifty feet or more into the ceiling. Workers wore coveralls and were outfitted with breathing apparatuses in order to protect the worker and minimize sample contamination. The core samplers were percussion driven steel tubes previously coated with plastic. The deposits directly under the crack were five feet, four inches deep and represented sixteen years' accumulation of guano. This cave had been previously mined for its guano deposits (mined to bedrock in 1956) and as a result, the age of the bottommost layer was accurately known. After the core sampler was driven down to bedrock, a trench was dug to expose the length of the core. The core was then removed and the ends sealed with sheet plastic and paraffin. The cores were then wrapped in 3" foam rubber and immediately transported to our laboratory for dating and analysis.

Once in the laboratory, the cores were placed in a horizontal die and the top half of the core sampler casing was cut away with a dental drill, exposing one half of the cylindrical guano core. Since there was some vertical mixing at the core sampler/guano interface, the top half of the exposed guano was mechanically removed by vacuum down to the center of the core. The core was then dated by a stratigraphic method described elsewhere (1).

After the annual layers were defined and dated, they were extracted with an acid washed ceramic spatula and transferred to 250 ml erlenmeyer flasks with 24/40 stoppers for weighing. Approximately one gram samples were digested with 25 ml of either 1:1:2 $HNO_3:H_2SO_4:H_2O$ or 42:4:4 $H_2SO_4:HNO_3:H_2O$ for 20 hours in a 40°C shaking water bath. Five percent $KMnO_4$ was added periodically to insure oxidation of the mercury during digestion. The samples were then removed from the water bath and cooled. Five additional milliliters of 5% $KMnO_4$ were added to each flask, 2 ml of potassium perchlorate and 2 ml of hydroxylamine hydrochloride completed the pretreatment. The samples were analyzed in five duplicate lots by cold vapor atomic absorption on a single beam Norelco Unicam Atomic Absorption spectrometer and a Laboratory Control Data double beam analyzer. The averaged results of five analyses are shown in Figure 1.

Discussion

Figure 1 shows a general zigzag pattern in the mercury concentrations found in consecutive years. It is also apparent that concentrations reached a relative maximum in 1957 and showed a large decrease in 1958 and 1959 and, in subsequent years, did not return to the high levels

Figure (1). Chronological record of mercury concentrations found in guano deposits of the Mexican free-tailed bat *Tadarida brasiliensis* recovered from the dry cave in Eagle Creek Canyon, Greenlee County, Arizona.

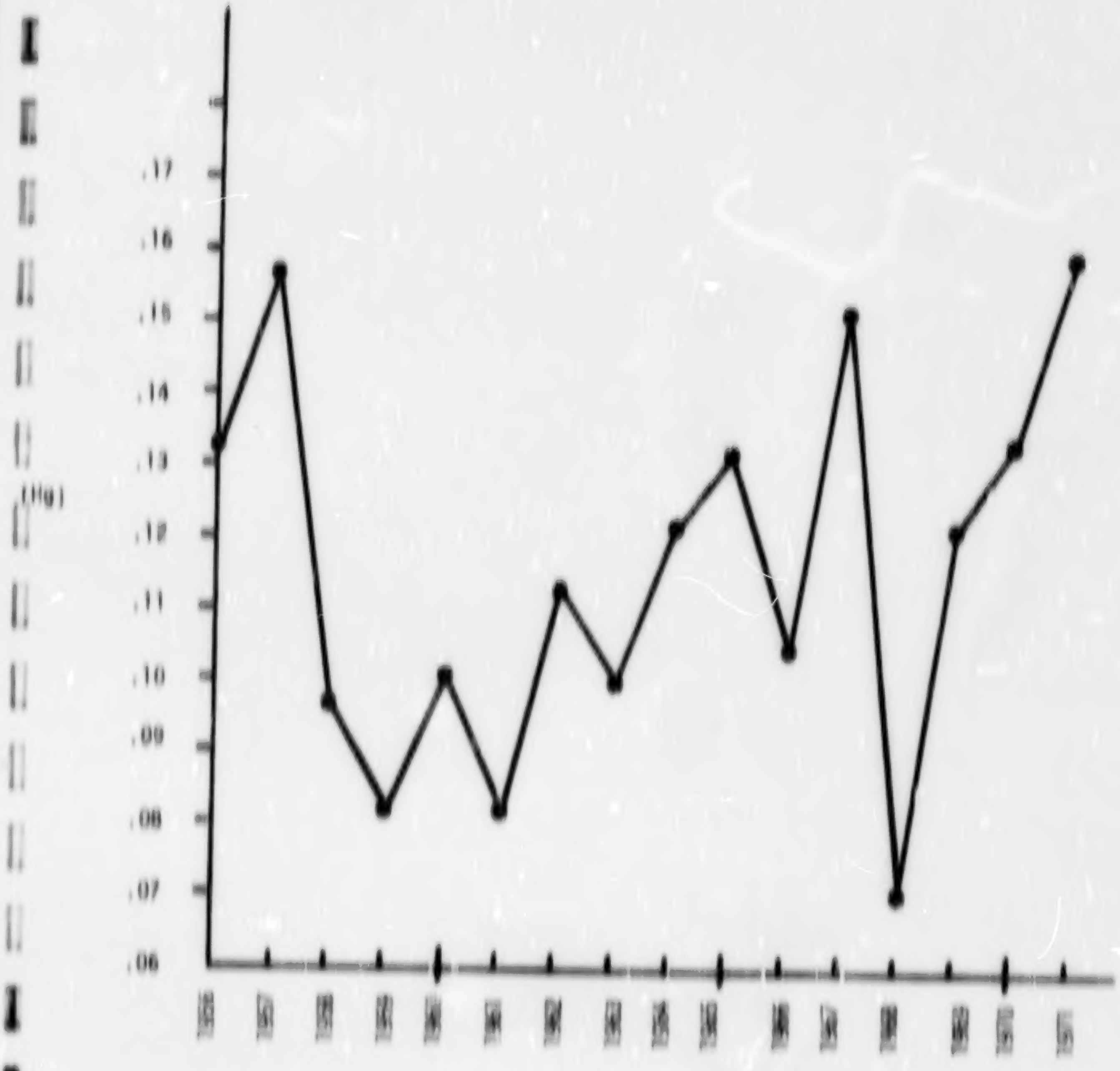
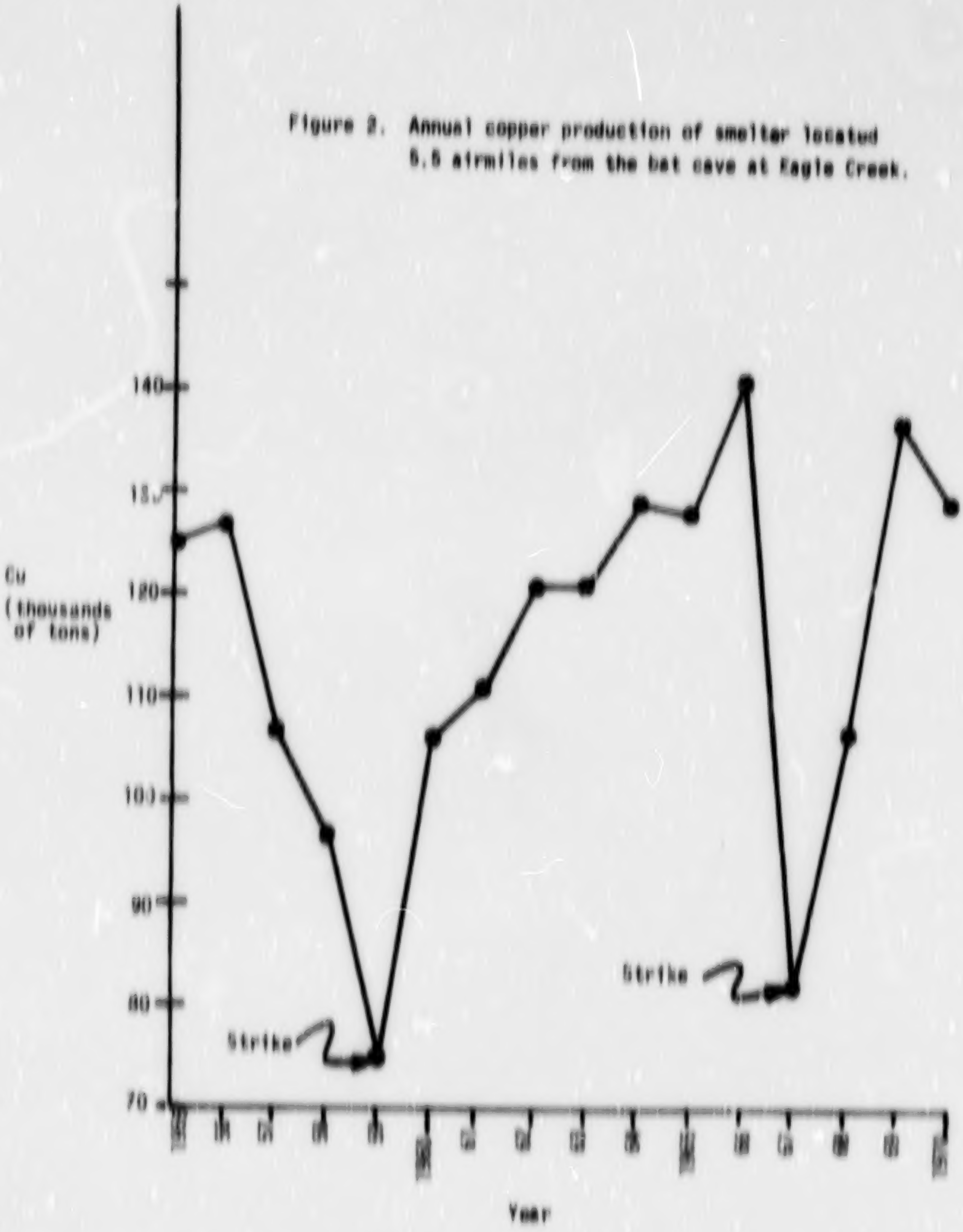


Figure 2. Annual copper production of smelter located 5.5 airmiles from the bat cave at Eagle Creek.



observed in 1957 until 1971. Figure 2 shows the annual copper production of the nearby copper smelter. Copper production decreased in the years 1957, 1958, and 1959, due in part to strikes at the plant. After a gradual recovery, the copper workers' strike of 1967 again depressed copper production until 1969 when copper production was back to normal. There is a one year delay observed between the decrease in copper production and the decrease in mercury in the food chain of the free-tailed bat, which implies an indirect route of entry into this mammal's food chain. If the animals were ingesting mercury in their drinking water, there should be no delay observed because the bats are present in the area during the rainy season at Eagle Creek when scrubbing of mercury from the air by rain should be maximal. This delay suggests that the mercury is taken up from the atmosphere either directly or from precipitation at a lower level on the food chain. Since the staple in this bat's diet is microlepidoptera (4), it is reasonable to suspect either the moth larvae or the vegetation they feed on as the port of entry into the bat's food chain. A more extensive investigation of the transport system of mercury into and through the food chain of the free-tailed bat is needed.

Conclusion

Fluctuations in atmospheric levels of mercury are observed in annually deposited guano of the free-tailed bat which correlate with copper production in the area. This substrate provides a unique chronological record of the extent of mercury pollution in an area. Thus, it has potential uses as an indicator substrate for evaluating the effectiveness of clean-up or pollution abatement procedures. Since this substrate is deposited in dry caves, it is not subject to leaching by percolating ground water. It may be useful for assessing the impact of recent geological phenomena, as well as soil tillage (surface altering activities), on levels of environmental chemicals.

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MOLYBDENUM AS AN ENVIRONMENTAL POLLUTANT

Gerald M. Ward

The official title of the project is "An Interdisciplinary Study of the Transport and Biological Effects of Molybdenum in the Environment." The project is supported by the National Science Foundation and the principal investigators are Dr. Willard Chappell and Dr. Roger Jorden of the University of Colorado. The individual investigators represent many disciplines, many departments, and two universities.

The project was initiated because the levels of molybdenum in certain waterways in Colorado were found to exceed the national average. This is probably not surprising since Colorado produces about 42% of the world's supply of molybdenum. The biological effects of molybdenum on man are essentially unknown but molybdenum toxicity in ruminant animals has been recognized since 1938. Molybdenum as a contaminant is not considered as hazardous as some other heavy metals such as mercury and lead but, unlike these elements, it has a much higher biological availability; it is more readily absorbed from the intestinal tract.

This project was designed to study the sources, transport and availability of molybdenum to man on the Eastern Slope of the Rocky Mountains in Colorado.

It was early established that the principle source of molybdenum was the Climax molybdenum mine at Climax, Colorado. The drainage from this mine area is through the Ten Mile Creek to Dillon Reservoir. Water from Dillon Reservoir is taken through the Roberts Tunnel under the Continental Divide into the South Platte River in order to augment the water supply for Denver. The South Platte flows through Denver and on through the farming areas around Brighton and Platteville where it furnishes irrigation water. Water from Dillon Reservoir is used intermittently and distribution to Denver residents depends upon their location in the city. The normal flow from Dillon Reservoir is through the Blue River to Kremmling and into the Colorado River.

Another source of molybdenum in the Denver Metropolitan area is Clear Creek which drains the area of another but smaller molybdenum mine near Empire, Colorado. Clear Creek supplies water to the towns of Golden and Westminster and irrigated farms in the region.

The relation between mining activity and the concentration of molybdenum in water is supported by the numbers below which, although maximum values in some cases, are representative of the situation found in Colorado.

Ten Mile Creek	1,000	grams/liter
Dillon Reservoir	300	
Blue River	240-430	
Denver Water (from Dillon)	190	
Denver Water (other sources)	2	
Golden, Westminster Water	550-580	
Mean United States Water Supply	0.35	

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It is clear that there are great differences in molybdenum concentration in water associated with mining and milling. It has been demonstrated that such high levels in streams are associated with human activity. Streams draining undisturbed areas of known molybdenum deposits have normal molybdenum levels.

Biological Effects

The biological effects of excess molybdenum were first described in England for sheep and cattle in 1938. In 1941, symptoms of molybdenum toxicity in cattle were described in the Blue River Valley where grazing cattle on irrigated meadows is the general practice. The clinical symptoms in cattle are severe diarrhea, poor appetite, and a gray or rusty coat color on dark colored animals. Osteoporosis has also been described in cattle. Molybdenum toxicity apparently results from a relative copper deficiency. Ruminants are 100 to 200 times more sensitive to molybdenum than non-ruminants such as horses, swine, poultry, or rats and presumably humans although we have no information.

The mechanism by which excess molybdenum intake causes a copper deficiency in ruminants is not understood. One suggestion is that a biologically inactive molybdenum/copper complex is formed thereby reducing the availability of copper. The level of sulfate in the diet is also an important factor because a high sulfate intake will cause a reduction in molybdenum uptake. Molybdenum is a structural component of the enzymes xanthine oxidase and aldehyde oxidase. These enzymes are required for uric acid metabolism and speculation would suggest a possible relation to gout.

Research Plan

The flora and fauna of the alpine regions is being sampled as well as the soluble and particulate material in the water courses. Comparisons are made between the molybdenum level in plants, invertebrates, mammals, and birds found in the undisturbed and in the mining areas.

A detailed study of molybdenum concentrations in the water and biota in Dillon Reservoir is underway because a marked seasonal turnover has been observed. The highest levels are found at the bottom during summer and the water for Denver is withdrawn at this level. The layering phenomenon may have a biological or a physical explanation. An extensive monitoring system has been set up to measure molybdenum in streams and rivers draining the mountains of central Colorado.

Agricultural studies are being conducted in the farming regions north of Denver that are dependent upon irrigation water, some of which contain the higher levels of molybdenum. Irrigation water, soil samples, and crops are being analysed to determine the transfer of the element. It is known that availability is greater from alkaline than acid soils. The availability from a variety of soils in this region is being evaluated by greenhouse studies.

Molybdenum is readily absorbed by cattle and appears in milk and as a result, provides a simple means of evaluating the amount in the feeds fed to cows. Since the forage fed to milking cows is still produced on many farms, a good transfer study can be established from irrigation water to soil to plants to milk. Preliminary studies indicate a range of 50 to 150 µg/liter for milk produced in the Denver area with some indication of higher levels in those areas dependent on irrigation water with higher molybdenum concentration.

By determining the molybdenum content of other important dietary components, it should be possible to estimate the dietary (including water) intake of average residents of Denver.

Metabolic studies are underway to evaluate the effects of low level (1-10 ppm) chronic intakes of molybdenum by rats. The effects of molybdenum on calcium metabolism and osteoporosis is being studied.

Analytical work is, of course, an important phase of this work because it is necessary to analyze a very large number of samples of diverse character with speed and precision. Methods under study include wet chemistry, x-ray fluorescence, and charged particle induced x-ray fluorescence (using the C.U. cyclotron). Data handling likewise is essential and is being developed to handle and retrieve all the data of the project.

An important aspect of the project is developing cooperation with many public and private agencies.

Public opinion regarding the possibility of molybdenum contamination is under study. A survey has been conducted to study public perception of environmental hazards generally and if warranted, a follow-up survey will be conducted.

The economic impact of molybdenum production of pollution possibilities and abatement has been prepared.

LEAD IN SOILS AND PLANTS

R. L. Zimdahl and J. H. Arvik

For the past two years, a research project entitled "Impact on Man of Environmental Contamination Caused by Lead" has been under way at Colorado State University. This project, financed by the RANN (Research Applied to National Needs) Division of the National Science Foundation, includes seven faculty investigators and several graduate students and technicians. The particular portion of the project with which this paper deals is the transport of lead through soil and its uptake by, and effect on, plants.

As part of the study of lead transport from a line source, to and through soil, a site with a traffic volume of 18,000 cars per 24 hours was selected on Interstate Highway 25, north of Denver, Colorado. We were interested in learning where lead emanating from automobiles was accumulating, and the rate of accumulation. The first work utilized a truck-mounted hydraulic probe to sample the soil in six-inch vertical increments to a depth of three feet, and in horizontal increments to approximately 500 feet from the edge of the interstate lanes. The 0 to 6 inch sample analysis (Figure 1) shows that lead concentrations were highest immediately adjacent to the roadway and concentration rapidly diminished with depth and distance. Measured lead values for soil samples obtained further than about 100 feet from the highway were indistinguishable from background. The baseline or background value of lead in soil typical of the field site location was determined to be 22.9 ppmw with a standard deviation of 4.4 ppmw (20). This value is somewhat higher than the world average of 16 ppmw (6). Lead values, shown in Figure 1, reached 530 ppmw on the east side of the northbound lane and 370 ppmw on the west side of the southbound lane. Average traffic flow rates for the two lanes were identical and the lanes were constructed and opened to traffic at the same time. Preferential build-up of lead in soil immediately adjacent to the northbound lane was confirmed by sampling at one-inch intervals from the surface to six inches. These results are presented in Figure 2. The data in Figures 1 and 2 confirm the observation made by others (2, 4, 11, 13, 17, 19, 24) that lead content decreases with increasing depth and distance from the highway. The mechanism by which preferential build-up occurs may involve reinjection of lead particles into the atmosphere from those originally deposited on the west side of the highway, with subsequent redeposition on the east side. However, our data do not strongly support this contention. A second hypothesis was that the prevailing wind direction might be from the west and account for deposition to the east. Wind speed and direction records, taken at the site, clearly indicate a predominant north or northwest wind source. Taken alone, these data support the finding that more lead is present along the east side of the highway, but they do not totally account for the unequal distribution. It is known that load conditions on the automobile can affect lead emission rates and some preliminary work suggests that a slight rise to the north may account for the observed differences. That is, the amount of gasoline a car uses as it goes by a given point or the load under which it is operating may be as important determinants of lead deposition as the number of cars going by that point (3, 7, 15).

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Lead Accumulation in Soil Adjacent to Roadway

144th St. Exit, Interstate Highway 25, Colorado, October, 1970

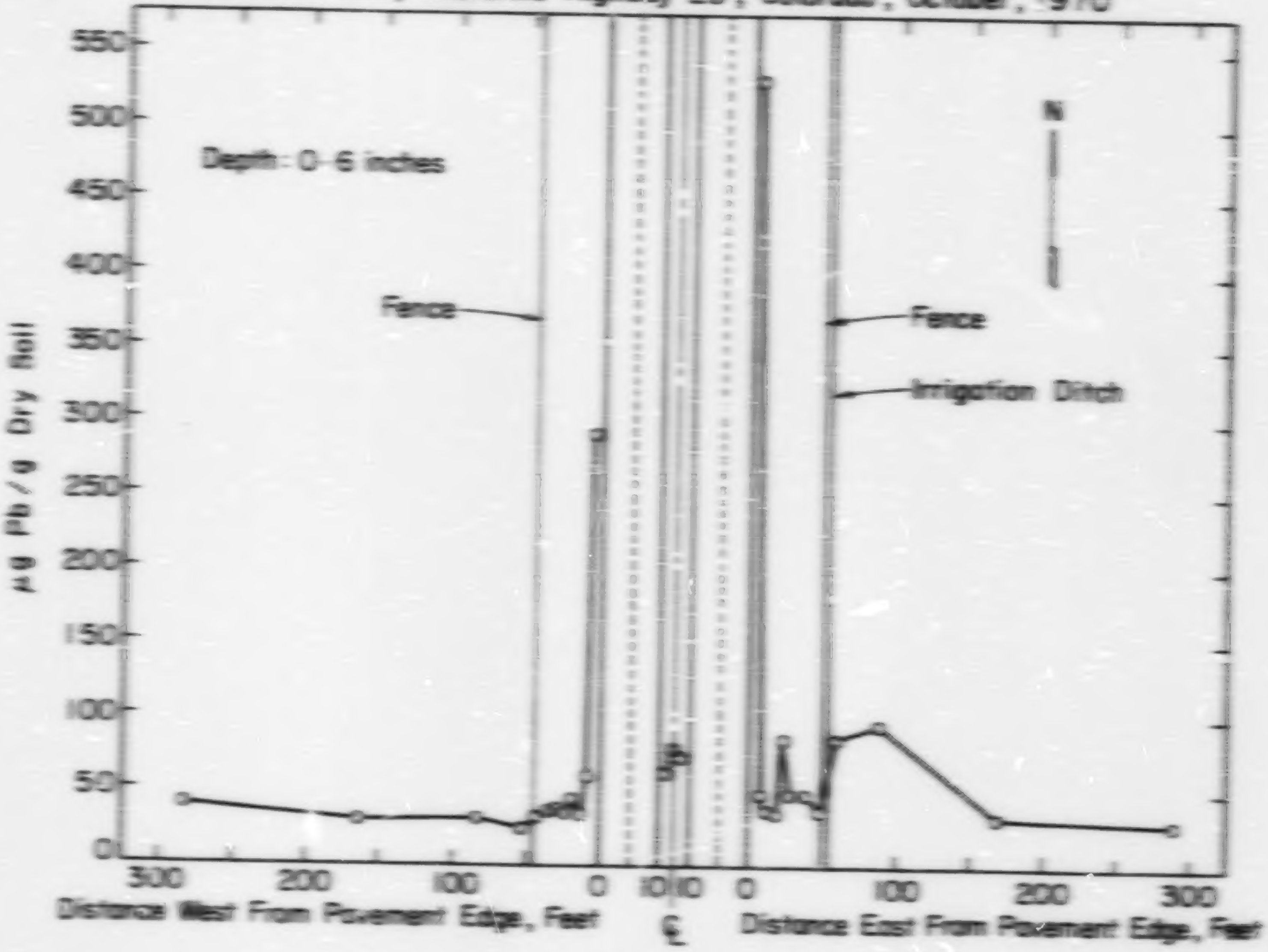

Figure 1

LEAD ACCUMULATION IN SOIL ADJACENT TO ROADWAY

140th ST. OVERPASS, INTERSTATE HIGHWAY 25, COLORADO, SEPTEMBER, 1970

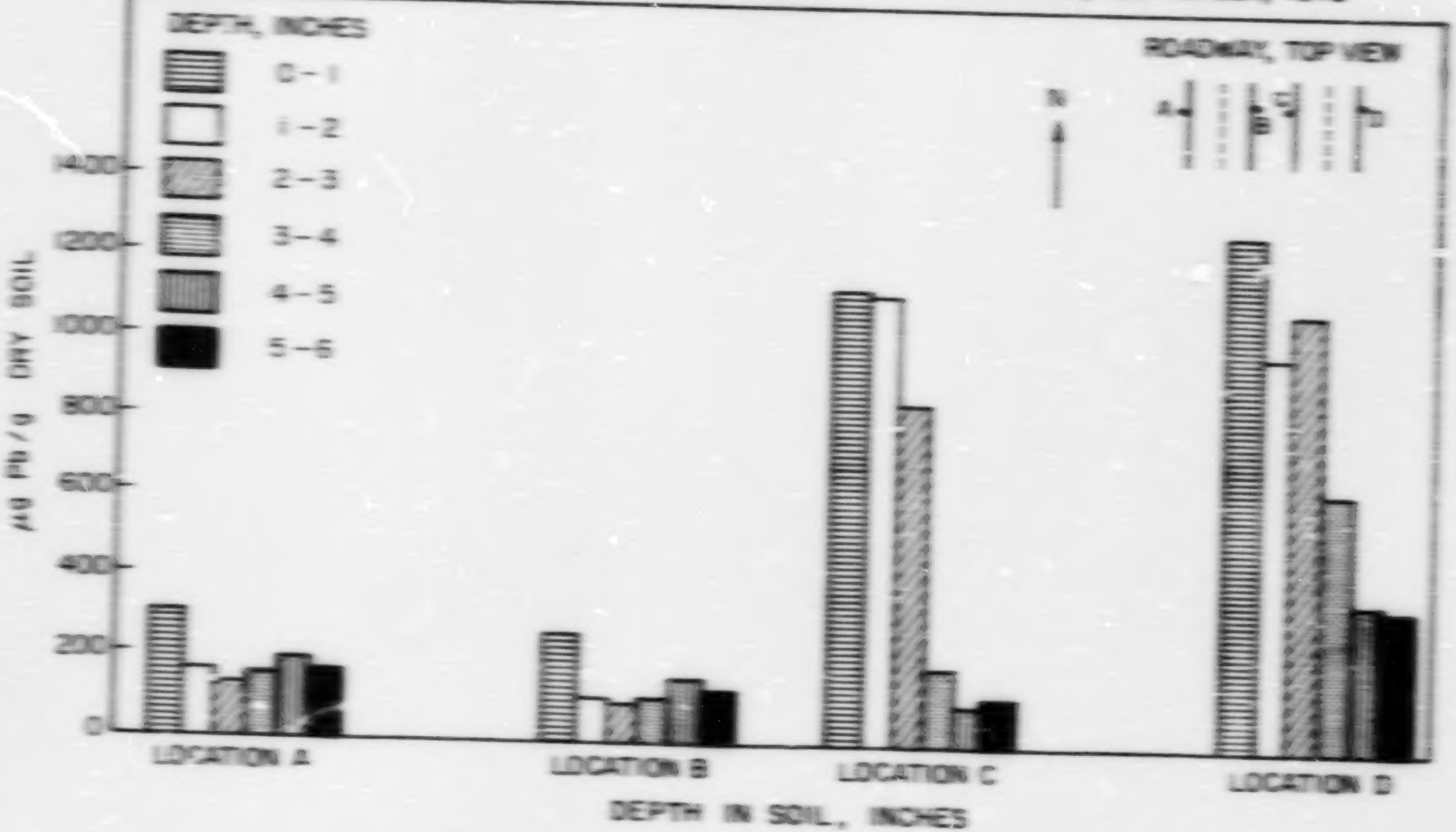

Figure 2

We were also interested in determining the capacity of the soil to sorb or immobilize lead. Our first experiment studied the reduction in the initial activity of radioactive lead (^{210}Pb) sorbed from solution as a function of time. We found that with San Luis, a fairly sandy soil, and Weld, a clay loam soil, the sorption equilibrium occurred in about 24 hours. Another experiment measured the rate at which lead was depleted from a lead nitrate solution by four soil types. The sorption equilibrium occurred in 24 to 48 hours. Knowing this value, we proceeded to study the sorption of lead by soil. The experiments were conducted by adding a solution of lead nitrate of known concentration to a known weight of soil and holding them at constant temperature in an oscillating water bath for 48 hours. The supernatant liquid was decanted and centrifuged and a 5 ml aliquot analyzed for lead.

A total of seven different soils have been used in these studies. They range from soil with 87% sand to a heavy clay soil. The cation exchange capacity (CEC) varies from 2.5 to 38. A plot of N* (the theoretical monolayer capacity) derived from the Langmuir sorption equation vs. CEC shows a positive correlation between the two. The exception to this generalization is found with soils that deviate markedly from a standard pH. Initially all of the soils used had a pH near neutrality. Another soil had a pH of 4.1 and did not fit the correlation between N* and CEC. We hypothesized that pH could be an operative factor and that raising the pH would bring N* into line with the other soils. This was done and our hypothesis verified. Thus, cation exchange capacity is a primary determinant of the ability of a soil to sorb or immobilize lead but the effect of exchange capacity is attenuated by pH. As we lower the pH of the soil less lead is sorbed to the soil and, therefore, may be more available to plants.

Lead in plants. Our initial studies were designed to measure lead uptake by plants growing in hydroponic solutions containing lead and in soils that had been doped with various levels of lead. We hoped to learn if different kinds of plants took up different amounts of lead and how much lead a plant could take up when growing in hydroponic culture. We have also studied the possibility of foliar uptake of lead. Sugar beets, corn, beans, and wheat grown in soil treated with lead nitrate take up more lead as the level of lead in the soil increases from approximately 80 to 2000 ppmw. Sugar beets seem to have a much greater ability to take up lead than the other three plants. These same plants have been grown using techniques of hydroponic culture. The plants are started and grown to a predetermined size in Hoagland's nutrient solution. They are then placed in a lead solution of known concentration. We have shown that lead uptake by the plant is directly related to solution concentration. Further, lead is concentrated in the root with limited translocation to the shoot. Analysis of pinto bean plants showed the greatest concentration in the root. The stem often had a level nearly equal to the root but never higher. The primary leaf was lower than the stem but always higher than the first trifoliate leaf. These observations held regardless of the beginning lead concentrations. Table 1 shows lead uptake from solution by four plants. These data indicate that massive amounts of lead can be absorbed by a plant. It is also of interest to note that the ratio of the amount in the root to that in the shoot decreases with increasing solution concentration. Limited translocation from root to shoot has been reported (8, 9, 10, 21, 22). These data indicate limited translocation under most conditions and that roots have a limited capacity to bind and hold lead. With higher solution concentration, translocation of lead is increased and the concentration in the shoot increases. The data indicate, but do not prove, that lead uptake may be a passive process or that lead may affect root cell membrane permeability. If the latter is true externally, it would also be true internally and account for the translocation seen with increasing concentration. We intend to explore this in greater detail.

TABLE 1

Lead Uptake from Solution by Four Plants

Plant and Part	μg/g Dry Weight Absorbed from $Pb(NO_3)_2$			
	100 ppm Pb	Root/Shoot Ratio	1000 ppm Pb	Root/Shoot Ratio
Pinto bean root	32,400	231.4	54,400	4.1
shoot	140		13,300	
Sugar beet root	54,300		84,800	
Wheat root	9,300	35.8	16,000	2.8
shoot	260		5,800	
Corn root	10,600	27.2	22,700	2.5
shoot	390		9,160	

One of our objectives has been to resolve the controversy in the literature concerning the site of lead uptake by the plant. Citations are available to support root (1, 14, 21, 23) or shoot (foliar) (5, 18, 19) uptake. Our work has shown that root uptake does occur. No reports have separated the supposition of foliar uptake from the possibility that lead may exist as a topical coating which may become embedded in or fixed to waxy leaf cuticles (12, 19). Two experimental techniques have been developed to study the problem. In the first, an exposure chamber with a square cross-section and a removable top and bottom was fabricated from ¼" plexiglass.

A lead aerosol is generated by aspirating a water solution or suspension of a chosen lead salt through 3" x 8" quartz glass chamber which is wrapped with resistance heating tape and insulated by ¼" asbestos. The tape is connected to a variable output transformer which allows adjustment of the chamber temperature. Air pressure for aspiration is provided by a laboratory vacuum pump. The aerosol is flash evaporated at 325°C in the heating chamber and driven through an 18" water cooled condenser to remove the water vapor. A portion of the lead salt remains suspended in the air stream as a particulate and is introduced to the plexiglass exposure chamber. The aerosol is composed of particles measuring less than 1 μ, simulating conditions existing downwind of heavily-travelled highways (7).

Plants grown in commercial vermiculite irrigated with Hoagland's solution are placed in the exposure chamber. Vermiculite was chosen to permit more efficient cleaning of plant roots following treatment and to prevent the possibility of lead uptake from soil. The surfaces of the pots are protected from lead settling on them by putting on a layer of soil with a high sorptive capacity for lead. The pots are placed on a layer of the same soil in the bottom of the chamber. This isolates the roots above and below a layer of soil. Therefore, any lead which gets in the plant must come from foliar exposure. Considerable work has been done to develop this technique and determine optimum operating temperature, flow rates, particle size, and lead aerosol concentration. The method does offer great promise for specific foliar exposure. However, this technique alone will not permit us to determine if lead is in the plant or on the plant. In conjunction with other procedures, it will allow the study of foliar penetration of lead.

We recognize the possibility of stomatal penetration but do not consider it an important route of entry for particulates. The primary barrier for materials to enter the plant leaf is the cuticle, and if lead is taken into the plant through the foliage, it must first pass through that structure.

To approach the problem of cuticular penetration, we have modified the methods of Orgell (16) and Yamada (25). A 3% solution of pectinase (Rohm & Haas Pectinol 10-M) or cellulase-hemicellulase mixture (R & H HP-150 concentrate) is prepared in deionized water and adjusted to pH 3. Leaf discs are vacuum-infiltrated with the solution and agitated gently in a reciprocating water bath at 30°C for 24-72 hours. The enzymes remove the upper and lower cuticles from the leaves, as a plant leaf is basically a sandwich of cells and vascular tissue held together by pectins, and having its surface covered by a waxy cuticle. Fruit discs serve as well to provide a cuticle for penetration studies. After removal from the discs, the cuticles are examined microscopically to insure that no physical damage has occurred. They are then washed in deionized water and fixed with silicone adhesive across the open end of a 15 x 100 mm polypropylene test tube. Five ml of deionized water is introduced into the tube through a small hole in the opposite end. This tube is then partially immersed in a larger polypropylene test tube containing 25 ml of 100 ppmw solution of lead salt. The tubes are adjusted to prevent hydrostatic pressure on the membrane. After 144 hours at 30°C in a stationary water bath, the lead concentration in the two test tubes is measured. Experiments have been conducted on Philodendron leaf, apple, tomato, and pepper fruit cuticle. Even under the extreme conditions of high lead concentrations and long exposure times, far less than 1% of the lead in the larger test tube is able to penetrate the cuticular barrier. These data question the assumption of foliar uptake of lead and we anticipate that the plants exposed to an aerosol will support this contention.

In summary, our work to date does not indicate imminent hazard from environmental contamination of soils and crops by automotive lead. However, the margin of safety is not reassuring, and there are definite indications that less dramatic effects do occur in man.

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HEAVY METAL POISONINGS IN ANIMALS

Arthur A. Case

Abstract

Heavy metal poisoning in animals is produced by overexposure to antimony, cadmium, chromium, cobalt, copper, iron, lead, mercury, molybdenum, nickel, thallium, tin, and zinc. Animals are usually exposed by eating or drinking the toxicant which contaminates their food or water. The term poisoning usually means acute or chronic intoxication leading to death of serious disturbance of health.

Low grade metabolic disturbance of growth, nutrition, and reproduction may be economically more important than the frank intoxications as such. Poisoning may be due to deliberate malicious action of man but is more likely to occur from errors in the use and management of poisons, improper waste disposal or storage of toxic substances, or ignorance regarding the presence of toxic chemicals in the environment.

Recent instances of metabolic disturbances in cattle due to the imbalance of natural trace or major elements in the flood plains below mining or other earth-moving operations have occurred in Missouri. Similar situations are possible in drainage areas below surface dump areas or where toxic substances may be applied in one area and wash onto other places during heavy rainfall. The toxic substances are deposited in the flood plains by the flood water and are then taken up by the vegetation growing in the area.

Heavy Metal Poisonings in Animals

Intoxications caused by overexposure to heavy metals are not included as legally reportable conditions. Data have to be based on personal experience to a large extent (1) or on reports such as recent papers by Aronson (2), Buck (5), and Zook (17, 18) concerning lead poisoning.

More animals are poisoned by lead than any other heavy metal according to recent reports (1, 2, 5, 17, 18). Lead compounds are commonly included in paint, putty, glazing compounds, plumbing fixtures, motor fuels; and there are many other sources which make lead the heavy metal most likely to be available in the environment.

Antimony is found in certain kinds of paints and enamels and certain medical agents. Both cadmium and chromium are electroplating metals and may be associated with other metal refining processes. Mercury has wide use in industry and is still in use as a fungicide under certain circumstances.

Thallium is a dangerous and widely distributed rodenticide now withdrawn from approved use, but still widely available in certain areas. Zinc may be an industrial hazard and with certain other elements (zinc phosphide) can be a dangerous toxic hazard to any kind of animal and man.

Low grade metabolic disturbance of growth, nutrition, and reproduction may be of greater economic significance than losses from the more dramatic acute lethal intoxications in cattle (1, 3, 4, 6, 9, 10). Barshad (3) described a copper-molybdenum imbalance in cattle in California. Britton and Goss (4) worked with chronic molybdenum poisoning in Nevada, and Davis (8) encountered a complex imbalance under Florida conditions which also involved interrelationships with phosphorus, copper, and molybdenum. Church (7) cites normal relationships of the major and trace elements as well as imbalance and the effect of such on ruminant nutrition. Case et. al. (6) and Ebens et. al. (9) have recently reported complex interrelations of anomalous trace elements in the geochemical environment associated with drainage from mining operations in Missouri. Cattle were affected in much the same way as described for cattle by Fleming et. al. (10). In all of the situations involving an imbalance of copper-molybdenum reported by the authors mentioned above, the ratios of copper to molybdenum varied considerably from those ratios given by the National Research Council as normal, balanced ratios for good health (12).

Jones et. al. (11) have reported still another interrelationship of trace elements involved in neoplasia of rabbits and man.

Selby et. al. (13) discussed the effects of trace elements on human and animal health and their observations agree in general with those expressed by Schutte (14) and the more recent definitive reference by Underwood (16).

Thompson et. al. (15) mention normal interrelation of both major and trace elements for cattle, especially Charolais.

In some instances, so many elements, both major and trace, are anomalous and the interrelationships so complex that it is impossible or impractical to do other than move the young cattle and breeding stock away from the contaminated environment to an area that is nearer to a natural balance of the elements.

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ADVERSE HEALTH EFFECTS OF TRACE MATERIALS IN THE ENVIRONMENT

G. J. Love

Abstract

Trace metals produced varied systemic effects, related to the total quantities absorbed from water, food and air. These effects cover a wide range of biologic responses ranging from increased pollutant burden to death.

Because ambient air concentrations of most trace materials are low, most effects are produced by long-term continuous or intermittent exposure. Hence, epidemiological studies would be difficult to undertake.

Studies of pollutant burdens provide an alternative method that is useful for monitoring environmental exposure, for indicating biologic response, for obtaining information relative to the establishment of environmental standards, for reference points for research, and for safeguarding recycling technology. The application of these techniques is demonstrated in the studies of lead, mercury, beryllium, and asbestos that have been of recent concern to the Environmental Protection Agency.

Introduction

The human organism is exposed every day to a variety of stresses which in toto exert considerable effect on its well-being. Some of these, such as temperature, humidity, or pathogenic agents, we accept as being natural; and even though we try to control, artificially, the effects of these factors, we give little thought to the idea that they might be eliminated.

The realization that some of the chemical substances to which we are exposed may produce significant stress on the human body has developed in relatively recent times. We now know, however, that a number of illnesses or conditions formerly thought to be restricted to industrial situations might be occurring in nonoccupational situations as well. These stresses we do think of as lending themselves to elimination and the fact that the illnesses could occur is sufficient incrimination to produce the pressure needed for enactment of legislation designed to protect the general public against the possibilities.

Environmental pollutants such as heavy metals or trace materials are more difficult to study than are the major gaseous pollutants; it is more difficult to develop control strategies against them because effects can vary so widely and total exposure is almost impossible to ascertain.

Whereas the major gaseous pollutants, with the exception of CO, produce effects at the point of contact, effects of trace metal pollutants are caused by the total quantities of the material ingested and produce systemic effects over a wide biologic spectrum (Figure 1). These effects can be death when exposure is at extremely high levels, or overt disease at somewhat less exposure. At the other end of the spectrum, the effect is restricted to an increased body burden. This is the initial response to exposure. Between the increased pollutant burdens and the overt disease is a broad area of altered physiologic activity, first of no significance, but at higher or continued levels of exposure, these alterations may become greatly significant.

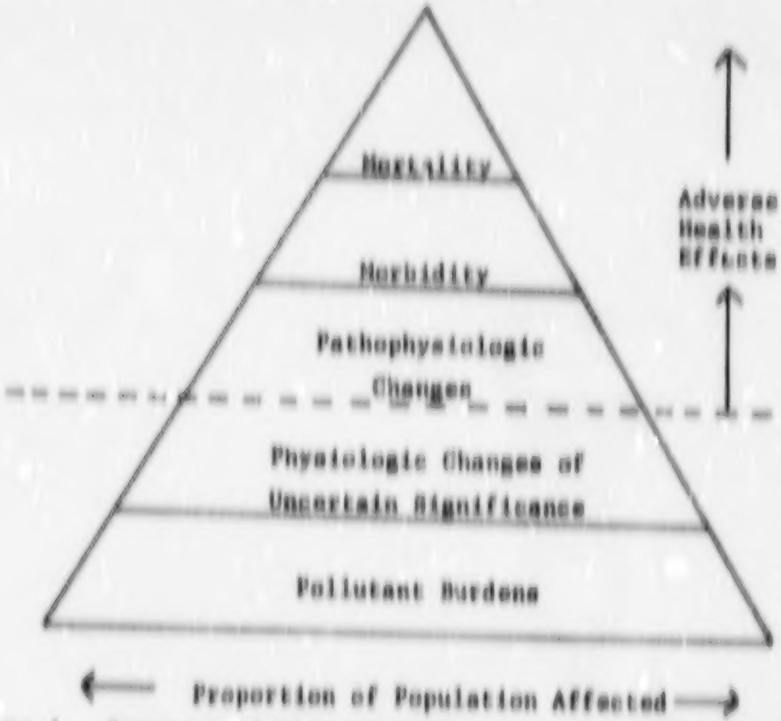

Figure 1. Spectrum of biological response to pollutant exposure.

Since ambient levels of trace metals generally are relative low and overt effects usually are produced by long-term continuous or intermittent exposure, epidemiologic studies are more difficult than usual to undertake, and they are very expensive. Primarily for these reasons, little information is available about the effects of chronic exposure to ambient levels of the pollutants. Most of the knowledge we have comes from occupational studies. On the other hand, the number of materials about which information is needed is growing daily, since several thousand new chemicals are developed each year and commercial uses are found for several hundred of these. The alternative to studying the mortality and morbidity that might be observed in the relatively small number of individuals indicated by the top of the response spectrum is to study the pollutant burdens indicated by the bottom of the biologic response spectrum which can be observed in many people.

By definition, a tissue carries a pollutant burden whenever it contains a residue greater than that needed for optimal growth and development; but in practice, measurement of pollutant burden means determining the total quantity present. Everyone has multiple pollutant burdens and these can serve as monitors of environmental exposure, as indicators of biological response, as inputs into environmental standards, as channel markers for research, and as safeguards for recycling technology.

Pollutant Burdens as Monitors of Exposure

Human tissues selectively absorb and store particular trace materials. Some tissue, such as hair, may actually record pollutant exposures for different time intervals. To utilize pollutant burden data for this purpose, however, it is necessary that the complexities of exposure and metabolism be understood. For example, our intake of cadmium involves air, water, food and tobacco smoke. All pollutant absorption is a function of exposure route, physical form and chemical composition. Lead fume is rapidly and almost completely absorbed from the lung, and lead bound to respirable particulates is largely absorbed. Conversely, lead bound to large suspended particulates fails to penetrate deeply into the respiratory tract and is removed by the cilia of the tracheal-bronchial tree to the throat and then swallowed. Absorption from the gastrointestinal tract is relatively limited. In addition, organic lead compounds are much more readily absorbed and are more toxic than are inorganic lead compounds. And finally, the distribution, storage and excretion kinetics generally are even more complicated than those of exposure and absorption. Biological half lives of pollutants differ greatly; for mercury it is 80 days, for lead 10 years.

Different tissues selectively concentrate different pollutant residues. Tissues with high lipid content like fat and brain serve as depots for Kr^{85}, alkyl mercury, chlorinated hydrocarbon pesticide, and polychlorinated biphenyl plasticizer residues. Liver and kidney retain most of the body cadmium, while lead, selenium and strontium concentrate in bones and teeth. Similarly, lung retains some pollutants including asbestos. However, when sampling living populations the epidemiologist usually must focus on more available specimens, which may be limited to hair, blood and excreta.

On occasion, human tissues preserved for various purposes have furnished insights into current pollution problems. Environmentalists have utilized museum specimens to gain further insight into pesticide, plasticizer and trace element pollution problems. There is a pressing need for human tissue banks to build an environmental flashback capability and we in the Environmental Protection Agency (EPA) are initiating such an effort during the current fiscal year to provide rapid information when needed relative to the several thousand metallic and synthetic compounds which potentially involve human exposures and human pollutant burdens. Not many years ago, infectious disease investigators encountered similar problems with unknown agents and set up a network of sera banks which have permitted them to rapidly assess the relationship of newly isolated microbes to older isolates, to disease syndromes, and to the factors of age, sex, race, residence and time. The tissue bank will provide a similar service for studies of nonbiologic etiologies. A coordinated tissue bank program perhaps must overcome obstacles inherent to standard tissue preservation; but freezing, lyophilization, and irradiation seem to be the methods of choice, with special care being devoted to containers.

Less dramatic but more useful tissue banks would involve easily preserved tissues like hair, deciduous teeth, and nail clippings collected from living populations whose environmental exposures are concomitantly measured.

Pollutant Burdens as Indicators of Biological Response

Human pollutant burdens can be related to the biological responses previously mentioned. These studies attempt to relate to response to tissue levels in responding members.

Studies in populations, however, must be interpreted very carefully. For instance, a given pollutant burden might represent a minor risk for an industrial population of medically prescreened adults and a major risk for susceptible groups in the general population. Susceptible groups include the pregnant mother and her fetus, infants and children, the elderly, and patients with chronic disease or other deficiencies. Age, sex or place of residence also may affect the extent of pollutant burden.

Pollutant Burden Input into Environmental Standards

One method of providing protection against environmental pollutants is the promulgation and enforcement of ambient air or emissions standards. Standards for pollutants characterized by direct exposure from a single environmental medium are set by considering control technology and the associations between pollutant levels and adverse effects and then employing a safety factor. Setting of standards for pollutants impinging upon man through multiple environmental media are much more difficult to derive. One input into such standards will be the pollutant burden of populations at greatest risk. For these pollutants, routine monitoring of population burdens can be at least as important as environmental monitoring. Population burdens may also delineate a priority order for standards development.

Also, critical evaluation of each standard established is necessary; and monitoring changes in human pollutant burdens is one way to determine whether standards actually are achieving environmental quality goals. Increasing pollutant burdens would warn that environmental controls are inadequate. Should population burdens approach levels associated with clinical toxicity, environmental standards would have failed and emergency action would be indicated.

Pollutant Burdens as Channel Markers for Research

Pollutant burden patterns can serve as channel markers for research, particularly in studies of chronic diseases or investigations of teratogenic, carcinogenic, and mutagenic hazards.

Pollutant Burdens as Safeguards for Recycling

In the past, control technology has focused upon disposal of waste products; but in the future, much more emphasis will be placed upon recycling, particularly for limited resources. The feasibility of recycling treated sewage water directly into municipal reservoirs, of consuming food products in whose growth or development solid wastes have been used, has been questioned by those who fear technology may not sufficiently safeguard the consumer. When recycling systems are utilized, appropriate human pollutant burdens should be monitored along with the recycled products.

To illustrate some of the ways in which pollutant burden information or the lack of it has created difficulties, let us consider some of the current responsibilities of the EPA. The first is related to the need to remove lead from gasoline. Lead is a ubiquitous substance, the toxicity of which has been known for many years.

We have been aware, for instance, of occupational hazards or the consequences of using pottery made from materials containing significant quantities of lead, when it is not properly glazed. But when it was decided that to control pollutants being emitted from automobiles it probably would be necessary to remove all lead from gasoline, it became necessary to assist with this effort by determining the extent of any health hazard that might be related to atmospheric lead.

Actually, most cases of clinically recognizable lead poisoning in the United States are included in three groups: (1) workers in lead trades, (2) people who consume illicitly distilled spirits which often consist of lead-contaminated alcohol, and (3) young children living in urban areas. There are adequate laws covering occupational exposures, and today, problems are rare. I understand that as taxes go up, the quantities of illicit liquor increase also. So the solution to that problem should be simple. The major environmental problem, then, is concerned with young children in urban areas. To try to determine just how much lead pollution contributes to this problem, it is necessary to make a number of assumptions.

The major source of lead for nonoccupationally exposed adults is the diet. The average daily intake from food is about 300 ug, with a range of 100 to 500 ug. Data indicate that this intake has not changed significantly in the past thirty years. The daily intake of lead from water has been estimated to be about 20 ug. Less is known about the dietary intake of lead by children, but the little information available suggests that daily intake in children can be significantly higher than 300 ug.

Only a fraction of the lead ingested in the diet is absorbed from the gastrointestinal tract. Lead balance studies in adults have shown that the average absorption over long periods of time is 5-10% of the ingested dose when intake is not excessive. Therefore, it can be calculated that in adults about 15-30 ug of lead are absorbed from the diet each day. The exact proportion of dietary lead intake that is absorbed by infants and children has not been determined, and it must be assumed to be similar.

During periods of normal intake, about 90% of the lead appears in the feces. A small amount obviously may initially be absorbed and then excreted back into the gastrointestinal tract. Most of the remainder of that not retained in the body can be found in the urine.

The contribution of airborne lead to the total amount absorbed each day has not been defined precisely, but some estimates have been made. Experiments with air containing 150 ug/m^3 of lead particulate matter with a mass median diameter of 0.25 μ showed that, in adults, 36% of the lead was deposited in the airways. It must be assumed, then, that a similar percentage of lead would be deposited when air containing concentrations of lead in the range of 1-10 ug/m^3 was inhaled, and that all of the lead deposited in the respiratory tract is absorbed into the body. From these assumptions, it can be predicted that the total daily absorption of lead from the air by a "standard man" engaged in light activity would range from 0.8 to 63 ug, depending on where he lives and works. The percentage of inhaled lead that is deposited in the respiratory tract of infants and children has not been determined experimentally; however, if it is assumed that the deposition is similar to adults (i.e. about 30%) and that the deposited lead is absorbed into the body, the amount of lead absorbed from the air can be estimated.

If other simplifying assumptions are made, a dose-response curve relating the concentration of lead in the blood to the daily absorption of lead from all sources can be constructed. For this purpose, epidemiologic data have been used which indicate lead concentrations in the blood of various groups of men whose estimated exposure to atmospheric lead had differed. The contribution of lead in the air to the daily absorption was estimated by using the assumptions mentioned above (i.e., that similar percentages of inhaled lead particulate matter are deposited in the respiratory tract, regardless of the concentration of lead in the inhaled air, and that all the lead that is deposited in the airways is absorbed). The amount of lead that will be absorbed from inhaled air is equal to the airborne lead concentration times the volume of air inhaled per day (about 23 m^3) times the percent of lead deposited in the respiratory tract. For example, if a person inhales air containing 2 ug of lead per cubic meter, and a conservative 30% respiratory deposition is assumed, then the lead absorption from the air can be calculated as follows: 2 ug/m^3 x 23 m^3 x 30% = 13.8 ug. An individual's total daily lead absorption can be calculated by adding daily dietary lead absorption (about 30 ug) to the amount absorbed from air.

Elevated blood leads and thus excess body burdens are associated with airborne lead levels greater than 2.0 ug per cubic meter. Increased urinary excretion of delta-aminolevulinic acid begins at blood lead levels of about 40 ug per 100 g of whole blood. Subtle signs of clinical lead poisoning have been associated with blood lead levels of about 50 to 80 ug per 100 g of whole blood. At the present time, there are no epidemiologic data which relate the lead concentration in the blood of infants and children to their estimated exposure to atmospheric lead.

On the basis of these data, a case has been made for the control of lead to protect the health of children in urban areas. This case argues that levels of atmospheric lead in excess of 2 ug/m^3 are accompanied by the following implications:

1. There is an increase in the accumulation in the body of a heavy metal with no known biologic usefulness but of known toxicity at moderately increased blood levels;

2. It can be shown that a substantial contribution to this body burden may come from atmospheric lead;

3. And finally, the question is raised concerning the consequences of an increased contribution to body burdens now found in urban residents. If the problem is pretty well restricted to children in these areas, there must be some factors peculiar to these areas. The argument must then acknowledge that at best, ambient atmospheric concentrations of lead are not directly toxic, but they may be indirectly toxic by increasing the risk of toxicity resulting from total intake of lead. Such indirect effects may relate either an added increase in the body burden of lead or to an increased risk of poisoning in young children who ingest excessive lead from other sources.

As far as other sources are concerned, we are familiar with the problem of lead toxicity associated with ingestion of paint chips containing larger quantities of lead; but there is another source that might be less significant yet should not be overlooked. This is dustfall in urban areas.

Dustfall in metropolitan areas contains a high proportion of lead, ranging from 1636 ug/g dust in residential areas to 2413 ug/g dust in commercial areas. Values up to 3397 ug/g dust have been collected close to heavily traveled roadways. These quantities may appear to be more significant when it is remembered that 1 ug/g is the equivalent of 1 ppm. These are the levels of lead in the dust found in city streets where most urban children play. So, while ingestion of leaded paint clearly is the principal cause of lead poisoning among children, it probably does not account for the approximately 25% of children who in mass screening programs in inner city areas did not appear to have clinical lead poisoning but had blood lead levels exceeding 40 ug Pb per 100 g blood.

According to a recent NAS report, dust may well account, in large part, for the higher mean blood lead content in urban children and the rather large fraction of these children whose blood lead content falls in the range of 40-60 ug per 100 g of whole blood, thereby bringing them into the range in which increased urinary excretion of ALA may be observed. Based on the evidence presented, it was concluded that airborne lead levels exceeding 2 ug/m^3 averaged over a period of three months or longer are associated with a sufficient risk of adverse physiologic effects to constitute endangerment to the public health of sensitive segments of our population. Since airborne lead levels in many major urban areas currently range from 1-5 ug/m^3, and since motor vehicles are the predominant source of airborne lead in these areas, attainment of the 2.0 ug/m^3 level would require a 60-65% reduction in lead emissions from motor vehicles.

EPA also has the responsibilities for designating hazardous air pollutants whenever it is believed that emissions from specific sources may endanger human health. Such designation requires the establishment of emission standards and designation of atmospheric levels at which the federal government will take emergency action. Three substances have been designated to date: asbestos, beryllium, and mercury; and the pollutant burden concept was prominent in the selection of recommendations for permissible ambient exposures or the significant harm levels.

Asbestos. The ability of asbestos to produce lung disease in the form of asbestosis has been known for many years. More recently, it has become apparent that asbestos exposure is associated also with the development of pleural plaques, cancer of the lung and digestive tract, and mesothelioma, an uncommon form of cancer. Both types of illness have been associated with the inhalation of asbestos fibers. Sufficient data have been collected to define what are believed to be reasonably accurate dose-response relationships for asbestosis. One consistent epidemiologic feature of the illness is that it develops only after many years of continuous heavy exposure and is not reported outside industry. The second type of illness has been shown much more recently to be associated with asbestos exposure and the epidemiologic relationships are not consistent. Cancer, especially mesothelioma, has been reported following exposures ranging from a few months to a few years and has been reported in persons living near asbestos mills as well as in factory workers.

The lack of dose-response relationships suggest that asbestos workers may not differ in their susceptibility to these malignancies from other segments of the population of the same age. The more susceptible individuals, even among the asbestos workers, may develop the illness as a result of short exposures to significant quantities of asbestos fibers or maybe under unusual exposure conditions not presently understood.

Present methods of sampling, identifying, and measuring airborne asbestos are not entirely satisfactory, especially if one is dealing with the low fiber concentrations that have been demonstrated in ambient air. The demonstration of ferruginous bodies and the positive identification of chrysotile asbestos fibers in lung specimens from persons with no occupational contact with asbestos indicates that these persons have inhaled and retained asbestos. Since there were no dose-response data available, EPA declined to indicate a "number" emission standard but is dependent for control on the maximum elimination of emissions. This, however, did not eliminate the requirement for indicating the significant harm level. This was derived in the following manner. A standard, proposed for occupational exposure to airborne asbestos, is a maximum of 2.0 asbestos fibers per cubic centimeter (cc) of air based on a count of fibers greater than 5 micrometers (>5 μm) in length, determined as a time-weighted average (TWA) exposure for an 8-hour work day, and no peak concentration to exceed 10.0 fibers/cc >5 μm as determined by a minimum sampling time of fifteen minutes. If the TWA exposure for an 8-hour day is converted to a TWA exposure for a 24-hour day, this would permit 0.6 fibers/cc >5 μm for 24 hours. A worker does not work every day in the year, and if it is assumed that he works approximately two-thirds of the days, 0.4 fibers/cc >5 μm is the TWA exposure for a 30-day exposure period.

Applying a safety factor of 2 to the above, the recommended imminent endangerment to health levels for asbestos above natural background levels for the general population are as follows:

1.0 fibers/cc >5 um TWA exposure for 8 hours
0.3 fibers/cc >5 um TWA exposure for 24 hours
0.2 fibers/cc >5 um TWA exposure for 30 days

Peak exposure to asbestos shall not exceed 5.0 fibers/cc >5 um as determined by a minimum sampling time of fifteen minutes.

Beryllium. When beryllium or its compounds is inhaled by humans or animals, it can cause various systemic diseases with pulmonary damage being of major concern. An acute chemical pneumonitis has been produced by inhalation of virtually all beryllium compounds. The total response seems to be dependent upon the degree, duration and type of exposure. Rapidly fatal cases have resulted from exposure to large concentrations of soluble salts in beryllium-processing industrial plants.

Most commonly, however, a chronic illness develops that is insidious in nature, developing as a dry cough and progressing to substernal discomfort and pain, general weakness and fatigue, and loss of weight. Attempts to correlate this chronic effect with time and duration of exposure have been unsuccessful. The delay in onset of manifestations can vary from months to many years. The illness is of long duration with a high mortality rate. Cases have occurred in persons exposed to beryllium in many forms in a wide variety of occupations and in persons not connected with the processing of beryllium but residing in the neighborhood of beryllium plants. The evidence also indicates that at least one member of a household of a workman employed in a beryllium plant contracted the disease from exposure to dust carried home on clothing.

Studies of chronic beryllium disease have demonstrated no simple dose-response relationships. Cases of chronic disease have occurred following exposures to low concentrations of a beryllium compound while no disease was produced at higher levels of exposure.

Thus, beryllium has been shown conclusively to be a hazardous substance even when exposure is to relatively low levels. Beryllium is unique in that there are three standards which stipulate, for different conditions, the permissible concentrations of beryllium and beryllium compounds in the air. One standard, designed to prevent acute occupational beryllium disease, sets a limit for peak exposures at 25 ug Be/m^3 for not to exceed 30 minutes. A second standard, designed to prevent chronic occupational beryllium disease, provides that in-plant atmospheric concentrations of beryllium should not exceed 2 ug/m^3 as an average over an 8-hour day. The third was proposed to prevent non-occupational chronic beryllium disease in communities around beryllium extracting and processing plants. This stipulates that the average monthly concentration of beryllium at the breathing zone level in the communities should not exceed 0.01 ug/m^3. Assiduous adherence to these standards apparently prevents new cases of beryllium disease.

Acute non-occupational beryllium disease has not been reported in the United States in the neighborhood of beryllium plants; therefore, there is no need to indicate a maximum or peak exposure level to prevent acute disease as long as the required lower level of exposure designed to prevent chronic disease is maintained. The non-occupational monthly mean limit of 0.01 ug/m^3 was based upon the report with 11 persons with non-occupational chronic beryllium disease in the area surrounding a beryllium-in-air concentration which produced the disease was greater than 0.01 ug/m^3 but probably less than 0.10 ug/m^3. The 0.01 ug/m^3 value was therefore established as the out-plant limit.

Recognizing that the scientific back-up of these levels is weak, it was believed to be more prudent to recommend that the level designated to represent imminent and substantial endangerment to health and therefore the level never to be reached in the ambient air should be 3 times the proposed out-plant limit rather than 2 times this limit, or an average monthly concentration of 0.03 ug beryllium/m^3 at the breathing zone level. This assumes that the 11 subjects used as the basis for the standard probably represented more susceptible individuals exposed to levels of beryllium in excess of 0.01 ug/m^3 and that chronic illness can result from exposures of little more than one month duration.

To protect non-occupationally exposed subjects in special situations such as observers at a rocket launch or residing in the vicinity of a test site, limits of 2.5 ug beryllium/30 minutes were selected to represent the absolute maximum permissible exposure. (2.5 ug Be/30 min. = .05 ug/day if for the remaining 23.5 hours exposure was zero.) This is one-tenth of the 30 minute maximum concentration permitted in industry and is selected arbitrarily as a level to protect the general population against acute effects. A 3-hour maximum permissible exposure of a mean level of 0.4 ug Be/m^3 should be imposed in addition to the 30 minute maximum.

The 30-day significant harm level of 0.03 ug/m^3 permits considerably less inhalation of beryllium than does the industrial TLV of 2 ug/m^3 for a 40-hour week which corresponds quantitatively to approximately 0.5 ug/m^3 continuous exposure. However, possible differences between the effects produced by continuous exposure and those produced by intermittent industrial exposure are not understood, and evidence indicates that sensitive individuals can develop chronic beryllium disease at continuous exposure levels not greatly in excess of 0.01 ug/m^3.

Mercury. Inhalation of air contaminated with mercury vapor or certain mercury compounds may produce intoxication or poisoning resulting from the absorption of toxic amounts by tissue in the respiratory tract. Furthermore, inhalation of mercury vapors or mercury compounds may be more detrimental to the body than is ingestion of these materials. Data suggest that absorption through the respiratory tract leads to a higher rate of accumulation of mercury in the brain than results from other routes of absorption. Once mercury passes the blood-brain barrier, it becomes more strongly bound in the brain than in any other organ of the body. The organic mercury compounds in general are more toxic than elemental mercury or its inorganic compounds.

Health effects related to absorption of mercury range from pneumonitis, gastrointestinal and central nervous manifestations in acute instances to a particular form of anxiety neurosis, erethism, the most characteristic result of toxic chronic exposure. No information is available on the minimum concentrations of mercury that will produce chronic poisoning.

Recently, considerable publicity has been given to the problem of mercury pollution in water. It has been learned that as a result of bacterial action, inorganic mercury can be converted to the more toxic organic forms and as such may be stored in biologic tissues. These concentrations can become greater and more significant as they move through a food chain until eventually some member of the chain can ingest a toxic dose. This bio-accumulation in food chains was documented first by the investigations of Minamata disease in Japan and subsequently in other parts of the world. How much atmospheric pollution with mercury may indirectly contribute to the levels of water pollution is unknown.

A safe level of atmospheric pollution with methylmercury or mercury vapor that could be established as a reasonable ambient air quality standard is 1 ug/m^3. This level is derived from the following data:

1. A daily intake of 300 ug Hg as methylmercury would be expected to produce at equilibrium a blood Hg level of 0.2 ug/g whole blood. This is the blood Hg level at which the onset of symptoms would be expected in the most sensitive individuals.
2. One-tenth of 300 or 30 ug Hg is selected arbitrarily as the maximum daily intake of Hg that should be permitted in the general population, thus providing a comfortable margin of safety.
3. The permissible intake of 30 ug Hg per day may be obtained from water, food, and inhaling 15-20 m^3 of air. At an air pollution level of 1 ug/m^3 15-20 ug Hg would be inhaled each day, leaving approximately one-third of the total or a maximum of 10-15 ug to be ingested in food and water.
4. Although there might be some argument about the accuracy of figures for the Hg content of food and water to arrive at the estimates for potential total intake, there probably is sufficient safety included in the recommended level to allow for a 100% error.

On the basis of these same data, it is recommended that the "significant harm" level of air pollution be established at 10 ug Hg/m for the following reasons:

1. If 300 ug daily intake can produce symptoms in sensitive individuals, this level of intake should not be permitted for even one day because no assumptions can be made about prior exposure for any individual.
2. In an area in which the atmospheric concentration of Hg is increased significantly, the quantities of Hg in food and water

might also be increased. Perhaps this would not be expected to occur immediately, but the possibility cannot be ruled out.

3. Consequently, if the "significant harm" level of Hg is considered to be a total daily intake from all sources of 300 ug, the maximum permissible contribution from air can be determined in the same manner used to estimate a safe air level.

4. This determination would indicate that a maximum intake of Hg from food and water might be expected to remain consistent at approximately one-third of the total intake of 100 ug at a time when total intake from air would be 100 ug Hg. If the total quantity of air inhaled each day is accepted as 20 m^3, this establishes the potential imminent endangerment level of Hg air pollution at 10 ug/m^3 24-hour mean.

5. This recommendation assumes that differences in toxicities of mercury vapor and methylmercury are not excessively great at lower levels of exposure and, therefore, the figure was derived as if these toxicities are equal. If subsequent research demonstrates that this assumption resulted in an overly-stringent standard, the level can be revised.

It is recognized that the 24-hour mean atmospheric mercury levels recommended here to be the level of potential significant harm within a population is the same numerically as the 8-hour industrial TLV for methylmercury. However, for the purpose of this recommendation, methylmercury and elemental mercury have been considered to be equivalent, when in reality there is little likelihood that atmospheric mercury will include significant quantities of methylmercury. It was believed that the assumption is necessary to permit applicable and timely measurements of atmospheric mercury. In industry, the TLV for elemental mercury is now 100 ug/m^3, but it is planned that this will be reduced to 50 ug/m^3.

The recommended significant harm level is considerably higher than the levels measured even in the worst situations within the United States. It is designed, however, to prevent for a single day the level of exposure that on a continuous daily basis is indicated to result at equilibrium in a blood mercury level of 0.2 ug/g blood if one-third of the total daily intake is obtained from food and water. A level of 0.2 ug/g blood is associated with the onset of symptoms in the most sensitive individuals.

ENVIRONMENTAL CHEMICALS AND CARCINOGENESIS

Hans L. Falk

The evaluation of carcinogenic hazards for man on exposure to environmental chemicals has been of very grave concern to agencies of the government, to investigators at universities and in industry, and through newspaper reports to the general public. The question whether a human cancer hazard exists has been answered to the best of everybody's ability in the positive or negative, depending on one's point of view. It has become painfully clear that relevant data on carcinogenic hazards for man have been sparse or altogether absent, and conclusions have had to be based on animal data. These studies even under the most careful planning and with the greatest precision have obvious limitations in the extrapolation of their results to man. In this paper, I will try to illuminate what is known and what is only surmised, taking DDT as an example, to explore the questions that have to be answered for an acceptable conclusion on carcinogenic hazards.

The simplest task in the evaluation of existing laboratory data for scientific adequacy of design and execution. It is here that science has its well-established rules, and conclusions can be accepted by all who utilize the scientific method. Nonetheless, even in laboratory experimentation and interpretation of results may be some room for heated discussion and disagreement. These are based on incomplete knowledge that is always encountered when a study is initiated and before hindsight prepared the way to easy criticism. However, many pitfalls have been recognized and remedied.

In testing a compound for its carcinogenic activity, it is often impossible to know its purity or the nature and amounts of impurities present in the available commercial product. It is, however, necessary to be aware of potential impurities but not to rely on the absolutely pure chemical for this first bioassay as one may otherwise miss hazardous substances which may only be present as impurities. The objection has been raised that one can never come to any conclusion if one is dealing with commercial products which with time may be altered in composition. This is a justified criticism and may lead to additional studies being carried out involving the pure components in order to get more information towards understanding this hazard.

Once we have reached acceptance of the chemical of choice and have obtained adequate information on its chemical and physical properties and impurities, it will still be necessary to concern oneself with the adequacy of the biological parameters of the study to be undertaken. Many of these are rapid tests and give essential information which serve as basis for decision for the long-term study to follow. The degree of absorption of the chemical from the environment needs to be established, be that through the lung, from the gastrointestinal tract, or through the skin and such studies must be followed up by others on retention and distribution throughout the body, together with rates of elimination which may depend on the metabolism of the compound. This information may be crucial in alerting us whether we have a hazardous substance in hand or whether the situation may never become serious.

For many chemicals, we have adequate information on purity and on the nature and extent of the impurities which are encountered. We are well informed of the stability and degradation of the compound in the environment; and we may know about its absorption, retention, storage, metabolism, and elimination from experimental animals. We can, therefore, answer adequately the important questions of whether the compound will be around long enough to be considered a potential hazard, or whether it can be destroyed and eliminated at a fast enough rate to be considered harmless. In the case of DDT, we know that its degradation is slow and, under certain conditions, may not take place at all, and that it can be absorbed and metabolized but that the process of metabolism in many species does not necessarily lead to a non-toxic compound. Although one of the major metabolites seems to be harmless for insects, in mammals DDE is quite toxic and possibly carcinogenic.

With these data in hand, one may be considered adequately equipped to undertake long range toxicological studies to determine if carcinogenic risk exists for future extrapolation to man. One may not as yet have adequate information on absorption, retention, and metabolism in humans until a spectrum of responses from different people has also been obtained. In the case of DDT, this kind of information is available; and for mammalian species, including man, qualitative differences in the handling of the chemical do not seem to exist.

Carcinogenic studies which have been carried out in the past have always involved a limited number of animals by necessity; and in order to obtain adequate information on tumor incidence, the dose to be administered had to be increased considerably, preferentially to a level where the chemical would not produce lethality or morbidity for an extended period of time. Such tolerance of the dose of test chemical is monitored by acceptable weight gain and lack of mortality for months while on the test chemical.

Although we may seem satisfied in general about the method of administration of the chemical, which is often oral administration, there are other parameters which present the basis for disagreement. These deal with the quality of the animal itself. It should be recognized that much is unknown about the test animal with regard to its weaknesses and susceptibilities. Many times, the choice of a particularly susceptible strain of animal is counter-indicated, while a particularly resistant strain may be recommended. This argument is often solved by recommending the choice of non-inbred animals as this would eliminate this impasse, whether the resistant or the susceptible strain is the most desirable for this study. The choice of the correct strain or species will depend on the specific condition that the experiment is to clarify.

With regard to quality of the diet, its stability and its contamination by pesticides, protection of animals against infection by viruses or bacteria or even deodorants and disinfectants that may be used in the laboratory and which may cause enzyme induction, thus changing the handling of the test compounds, experiments must be controlled. The results of these studies then will be utilized to determine whether a carcinogenic hazard may exist for man. In the case of DDT, it must be concluded that for mice, at least, a carcinogenic hazard does exist.

The problems we encounter in extrapolation from one species to another are based not only on differences in susceptibility for which we often have no information, but also on the comparability of the mode of administration and the dosing. These findings can be utilized to argue in several directions and frequently it is the decision founded more on the emotional rather than the scientific basis that prevails. As there are few data on environmental chemicals causing cancer in man but quite a collection of experimental data concerning conditions of exposure that have resulted in the production of cancer in animals, these facts can be reviewed to see how closely they correspond to the human exposure and what early signs of a parallel toxicity can be detected which might give us some confidence that parallel events have occurred.

For pesticides at very high dose levels, responses can be observed on the neurologic basis which may be considered similar for many species. At a lower dose level, no toxicity may be observed in any species, but a level of intake may be reached when a completely different type of toxicity is seen. It is referred to as the level of enzyme induction. This effect, generally considered as advantageous and helping in the detoxification of foreign chemicals, has been detected in humans as well as experimental animal species and shows considerable similarity in all species. It has been observed in the liver, the gastrointestinal tract, the lung, the skin, and other organs. With certain chemicals, this beneficial effect of facilitating detoxification backfires, and metabolism yields a more toxic product. It may also cause the rapid destruction of a compound needed for its biochemical activity, as happens in the case of certain drugs or steroid hormones.

It is generally assumed that on the cellular level, processes of detoxification may be parallel in specific organs of many species, but it should not be implied that these similarities are quantitative. Often, the same enzyme systems have been detected and the same metabolites have been identified, but there is no certainty that under all circumstances, the fate of the foreign chemical will be the same and that in the human, metabolism will lead to quantitatively similar end products. We know from experience with drugs and foreign chemicals that we do not obtain the same level of detoxification or the same rate of excretion and thus the same degree of toxicity in otherwise quite comparable species or individuals. Thus, although we have acceptable data on the carcinogenicity of various environmental chemicals, we must exercise care in interpreting the results by including well-documented variations in human metabolism and specific variations in response in this deliberation. The median human response does not cover enough of the total population at risk.

As pesticides have been of particular interest to many investigators during the last ten years, data on carcinogenicity of these compounds will be quickly reviewed. Such a review must be based on several factors: First, the persistence of the chemical will determine whether only the manufacturing and dispensing worker will be at risk or if the ultimate consumer may also be affected, even though he may not be aware of any exposure.

Since no epidemiologic findings suggesting a carcinogenic hazard have been associated with workers handling carbamate or organophosphate insecticides and since they are comparatively unstable so that they do not tend to persist for a long period of time, these compounds may not present a carcinogenic hazard for the general population on long range exposure to products that have in the past been treated with these insecticides. For the stable or poorly degraded insecticides, human epidemiologic data which would suggest a carcinogenic hazard are also unavailable; but because of their environmental persistence, animal experiments have been carried out; and for this group of compounds, positive data were obtained. This applies to some extent to most of the chlorinated hydrocarbon insecticides tested where on chronic feeding of high dose levels, the liver gave rise to hepatomas. Adequate dose-response data have not been obtained in most cases; but in each study, the level tested was the highest level that was tolerated over an extended period of time without weight loss. A number of pesticides have been singled out as presenting potential hazard by the realization that with increased usage, more pesticide could be stored in tissues of animals and man and a level could be reached which may be critical for the production of tumors. In the Report of the Secretary's Commission on Pesticides and Their Relationship to Environmental Health, pesticides have been subdivided into groups representing those that should be kept out of foods, those that should be further investigated, and those that apparently represent no hazards. They are listed in Tables 1 - 3.

TABLE 1

Compounds judged positive for tumor induction on the basis of tests conducted adequately in one or more species, the results being significant at the 0.01 level.

Registered for use on food crops	Registered, but not for use on food crops
* Aldrin	Amitrole
Aramite	Avadex (Diallate)
Chlorobenzilate	Bis (2-chloroethyl) ether
p,p'-DDT	N-(2 hydroxyethyl)-hydrazine
* Dieldrin	PCNB
Mirex	
Strobane	
Heptachlor[1]	CHLORDANE

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[1] Assigned to this group because a metabolic product, heptachlor epoxide, was judged positive for tumor induction, results being significant at the 0.01 level.

TABLE 2

Compounds yielding an increased tumor incidence significant at the 0.01 level but considered less tumorigenic than the mean of a group of positive controls. These compounds have first priority for additional testing.

Registered for use on food crops	Registered, but not for use on food crops
p,p'-DDD	Azobenzene
Monuron	CCC
Perthane	Chloranil
Piperonyl butoxide	Cyanamide
Piperonyl sulfoxide	Vancide BL
	Zectran

TABLE 3

Compounds yielding an increased tumor incidence significant at the 0.05 level. Similarly, they were concluded to be less tumorigenic than the mean of the same group of positive controls. These compounds have second priority for additional testing.

Registered for use on food crops	Registered, but not for use on food crops
Biphenyl	Genite-R99
Captan	IPC (propham)
2,6-Dichloro-4-nitroaniline[1]	
Gibberellic acid	
2-mercaptobenzothiazole	
Ovex (Chlorofenson)	

[1] Active ingredient in the formulation sold as Botran.

TABLE 4

Compounds yielding an increased tumor incidence in comparison with the negative controls but the level of significance was less than 0.02, possibly because too few animals were observed. These compounds have third priority for additional testing.

Registered for use on food crops	Registered, but not for use on food crops
a-(2,4-Dichlorophenoxy) propionic acid	1-Naphthalene acetamide
2-(2,4-DP)	2-(2,4,5-Trichlorophenoxy) propionic acid
2,4-D isopropyl ester	
n-Propyl isome	
Pyrethrin	
Zineb	

When reviewing the groups of compounds in each class, it is apparent that not all members of a group are mentioned, suggesting that there is no close structural correlation between carcinogenicity and chemical structure. This may be due to differences in toxicity so that different dose levels had to be administered, lack of testing of the compound so that data are not available, change in experimental design, species, strain, or length of exposure or observation which have to be taken into consideration if such a comparison is to be attempted.

The data that were collected in one large scale study were not meant to be the ultimate test before extrapolations would be attempted but were designed to be the first preliminary suggestions of hazards which must be studied in more detail and in different laboratories before conclusions can be drawn about legislative action to be contemplated. Only because a hazard to man's environment was demonstrated, actions were initiated on some of these pesticides before adequate information on carcinogenicity was available. In the case of DDT, additional experiments have been carried out which confirmed the earlier observations and thus justify action already taken to curtail the use of this compound. With regard to other pesticides which have proven to be tumorigenic to test animals, additional experiments are needed and following the Report of the Secretary's Commission on Pesticides' recommendations, are, I am sure, being carried out at this time.

TABLE 5

Compounds which were tested appropriately in one species only and judged not positive in that species. However, current guidelines for testing required negativity in two species. These compounds have fourth priority for additional testing.

Registered for use on food crops	Registered, but not for use on food crops
Atrazine	ANTU
2,4-D	Cacodylic acid
2,4-D Butyl ester	Copper 8-Hydroxyquinoline
2,4-D Isooctyl ester	Dicryl
Dehydroacetic acid	Diphenatrile
Dichlone	Dowicide-7[1]
Diuron	Hercules 7531 (Norea)
Dodine	Isolan
Orthophenylphenol	Karathane
Endosulfan	Pma; Phenylmercuric acetate
Ferbam	2-Sec.-butyl-4, 6-dinitrophenol
Folpet	2,4,5-T
Glyodin	
Maleic hydrazide	
Maneb	
Methoxychlor	
Methyl Zimate (Ziram)	
Nabam	
Phenothiazine	
Planofix: N.A.A.	
Propazine	
Simazine	
Tetradifon	
Thiuram (Thiram)	
Tillam-6-E	

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[1] While Dowicide-7 is registered for nonfood uses, its use in food containers and packaging materials is permitted under registration. Wooden fruit (berry) boxes may contain up to 50 ppm.

CHEMICAL INTERACTIONS

Frederick W. Oehme

The subject, "Chemical Interactions," is one that has only recently attracted attention amongst health scientists. However, the importance of chemical interactions far exceeds the limited emphasis given to it and the even smaller body of knowledge available about this biological-interchemical syndrome.

Most health and environmental scientists are accustomed to dealing with individual foreign chemicals and the effect they might have on any given biological system. We are trained to recognize the potential health hazard, clinical signs and lesions, and treatment and control recommendations for specific intoxications, such as lead poisoning, organic-phosphate poisoning, or nitrate toxicity. Few of us, however, recognize the multiple problems that may occur if two or more foreign chemicals are present and affecting the body systems at one time. The frequency with which combination drug therapy is employed in human and animal medicine and the wide variations of environmental pollutants to which man and animal are continually exposed give ample opportunity for multiple biological-chemical reactions and interactions to occur. Indeed, such adverse effects have been occurring with increasing frequency, and the widespread availability and use of such chemicals makes consideration of their health hazard of importance.

The purpose of this paper is to review the problem of chemical interactions by discussing the physiological and chemical factors determining how toxic agents exert their effect, suggesting proposed mechanisms for toxic responses due to chemical interactions, and documenting implications of such responses and suggested means for health scientists to become more aware of and combat this growing diagnostic and therapeutic problem.

Factors Affecting Toxicity

Even the beginning scientist is aware of the normal distribution curve and the fact that certain individuals in a population may demonstrate greater or lesser than normal responses to given chemicals; however, the vast majority of a given population will demonstrate a response closely grouped around the mean for a given set of circumstances. These circumstances--those that limit variability in response--are themselves subject to variation and with fluctuation in circumstances will result in increased or decreased toxicologic effects.

The commonly cited factors affecting toxicity are listed in Table 1. The dose or amount of toxin received is the single most important variable; but practical circumstances and the increasing production of new foreign chemicals makes all the factors significant. The toxin's physical and chemical properties determine whether significant amounts will be absorbed. The route of exposure affects absorption and the rapidity of onset of action. Absorption of the toxin is largely determined by its lipid solubility, but once absorbed, the biotransformation of the chemical is a vitally important process by which primarily the liver detoxifies

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the material. Distribution throughout the body, metabolism by enzymes, possible accumulation in body tissues, and the ease or lack of elimination from the body are all additional equally important variables. Of the factors relating to the animals involved, species differences are most important. Anatomical and physiological differences are obvious, but most critical are the variations in enzyme levels and the biological capacity for detoxification of foreign chemicals. Size, age, and sex of the exposed animals are also important variables, with the very young and old being more sensitive than the healthy mature individual. Females of the species are usually more susceptible to intoxication than males. Animals in poor health or with pre-existing liver or kidney disorders are more adversely affected by foreign chemicals than are individuals in full health.

TABLE 1

Factors Affecting Toxicity

1. Amount of toxin received
2. Physical and chemical properties of toxin
3. Route of exposure
4. Absorption of toxin
5. Biotransformation of toxin
    a. Distribution
    b. Metabolism
    c. Accumulation
    d. Elimination
6. Species involved
7. Size, age and sex of exposed animal
8. Health of animal
9. Individual variations

Even within groups of the same age, sex, and health, and receiving the identical chemical via a uniform route, variations still occur in a population. These are termed "individual variations" or idiosyncrasies. While many of the factors producing individual variations are still unexplained, it is felt that genetics and the inherent capacity for detoxifying or metabolizing foreign chemicals play a large role in defining these unexplained idiosyncrasies.

Biological Detoxication

All individuals have mechanisms to detoxify and metabolize foreign materials. These are normally existing systems present to various degrees in all animals to modify the effect of an absorbed chemical. They are outlined in Table 2.

TABLE 2

Biological Detoxication

1. Physiological detoxication
    - a. Vomiting, diarrhea
    - b. Bind to protein, store in tissues
    - c. Excrete in breath, secretions, bile, urine
2. Biochemical detoxication
    - a. Enzymatic attack to increase excretion
    - b. Liver, kidney, intestinal mucosa
    - c. Make easier to detoxify further, make more water-soluble, split or destroy
    - d. Most detoxication decrease toxicity or inactivate

Foreign chemicals may be detoxified physiologically by the animal vomiting or developing diarrhea following ingestion of the chemical. Both responses serve to clean the digestive tract of the toxic material. Following absorption of the chemical, it may be bound to protein in the blood or tissues. The foreign chemical may be stored in inert and inactive forms in body fat or bone. Physiological excretion of the material from the body may be by elimination via the breath, body secretions, bile, or urine. Excretion by the kidney and urine is the most common means by which the body rids itself of a foreign chemical.

The second major mechanism for detoxifying chemicals is via enzymes found throughout the body. The purpose of enzymatic attack is to increase excretion and thereby decrease body levels of the chemical. The liver, kidney, and intestinal tract mucosa are especially high in detoxifying enzymes, but all tissues in the body are capable of metabolizing foreign chemicals to varying degrees. The goals of biochemical detoxification are to modify the chemical so that it may be excreted more easily in the urine, or to split or destroy the active form of the chemical so that it is no longer capable of producing its toxic effect. While the vast majority of detoxification steps decrease toxicity or cause inactivation of the foreign chemical, enzymatic attack occasionally results in increased toxicity through the production of metabolites that are more toxic than the parent compound.

Species Differences

It was mentioned earlier that anatomical, physiological, and biochemical differences between species of animals are major factors in determining the effect of a foreign compound on these animals. Some anatomical and physiological differences between species are outlined in Table 3.

Differences in digestive tract anatomy result in variations in absorption rates in various regions of the gastrointestinal system. Animals with relatively long digestive tracts have increased exposure time for absorption. Species with large numbers of microorganisms in their digestive tract, such as ruminants having microorganisms as an important part of their digestive process, have a huge number of bacteria and enzymes in the digestive tract capable of acting upon ingested chemicals. Excretory ability varies with the degree of activity of various excretory systems. The milking animal is more capable of excreting toxins soluble in milk than are animals not possessing this excretory process. Not only are the types of system available important, but the degree of activity of each system is also critical. Animals with limited urine output would be restricted in their capacity to excrete a toxin for which the kidney is an important excretory route. The pH of urine varies with animal species and affects the water solubility of the toxin and its capacity for reabsorption by kidney tubules versus excretion in the urine by maintaining water solubility. Species with large amounts of body fat, such as swine, are capable of storing toxic materials in an active form in their tissues. Animals having limited fat would not possess this safety factor. Dietary constituents affect the pH of urine and thereby the degree of excretion possible via the kidneys. In addition, dietary constituents present in the digestive tract may interfere with or enhance the absorption of foreign chemicals. Certain species of animals are subjected to excessive physical activities and other stresses which may alter the metabolism of absorbed toxins. A racing animal with a high metabolic rate will have a high rate of detoxification; individuals of some species subjected to physical stresses will have altered liver and kidney function, and detoxification of foreign chemicals may be decreased.

TABLE 3

Physiological Species Differences

1. Absorptivity in various regions of the digestive tract.
2. Length of the digestive tract.
3. Presence of bacteria, enzymes in digestive tract.
4. Excretory ability.
5. Methods of excretion possible.
6. Urine volume and pH.
7. Amount of fat in body.
8. Constituents of diet.
9. Physical activity and other stresses.

Biochemical differences among species (Table 4) are due primarily to enzyme variations. These enzyme differences are not only important because of the differences in digestive tract organisms and their enzymes, but also because of variations in the level of circulating enzymes and in the activity of enzymes in the liver and kidneys. In order to deal with such variations specifically, one would have to identify the specific biochemical pathway important for metabolizing the foreign chemical under consideration and then determine the activity of that pathway in the specific exposed species of animal. This is a complicated and time consuming process; hence little information of this nature is available for each of the common animal species, excluding man. However, investigations in this area are fruitful and it is potentially one of the most dynamic and rewarding areas for biochemical and toxicological research. Comparative studies are not only important in infectious diseases, but they are being seen as of at least equal value for chemical disorders.

TABLE 4

Biochemical Species Differences

1. Digestive tract enzymes
2. Level of circulating enzymes
3. Liver enzymes
4. Other degradative processes

Adverse Effects

Although "poisoning" usually refers to an acute overdosage or severe response to a foreign chemical, there is another area, "adverse effects," that is much broader in definition and may include poisonings. Table 5 presents a listing of some common adverse effects.

TABLE 5

Adverse Effect vs. Poisoning

1. Poisoning refers to acute overdose or sensitivity.
2. An adverse effect is any one of a number of additional reactions, which may include poisoning.
    a. "Side effect"--other than expected response
    b. Immune reaction
    c. Interaction with other compounds
    d. Enzyme inhibition or induction
    e. Carcinogenesis
    f. Mutagenesis

The commonly referred to "side effect" is usually nothing more than a biological reaction to a foreign chemical that is different from that normally expected. This reaction may be a form of toxicity or it may be an exaggerated physiological response due to reactions with receptors or body tissues other than the normal target organ. Adverse effects may be due to antigen-antibody or immune reactions. Sensitivities to foreign chemicals and anaphylactic reactions fall in this category. Chemical interactions are capable of producing a wide variety of adverse effects by blockade or interaction with receptors, alterations in enzyme activity, interference with enzyme systems, or by producing cellular reactions resulting in altered organ function and chemical elimination.

Enzyme inhibition or induction is a recently found but well-documented syndrome (1,4). This very interesting series of reactions results in decreased or increased enzyme activity due to concurrent or prior exposures to foreign chemicals. Inhibition is due to chemicals blocking enzymatic pathways and reducing the rate of metabolism of foreign compounds; hence prolonged response or toxicity due to the toxic material results. Induction results in increased enzymatic activity, with increased metabolism of not only the previously administered chemical, but also of other foreign compounds metabolized by the same enzymatic pathways. The result is more rapid biotransformation and excretion, a shorter biological response, and usually decreased toxicity. Excellent detailed descriptions of these phenomena are available to interested scientists (1,4-7).

The development of cancer and the production of congenital anomalies are two subtle but extremely important adverse effects that may result from exposure to foreign compounds. Both are difficult to reproduce experimentally, and clinical evidence incriminating specific chemicals in either condition is usually vague and, at the best, suggestive. The factors involved with cancer production are incompletely described (1), but the current national interest in this increasingly common health problem has resulted in the establishment of a national program to deal with cancer. Not only is the National Cancer Institute being rapidly expanded, but a major portion of the research effort at the newly developed National Center for Toxicological Research is devoted to the study of potentially carcinogenic chemicals. There is no doubt that a massive attack is being mounted on the problem of cancer; how productive it will be remains to be seen.

Mutagenesis and Teratogenesis

The appearance of birth defects is the outward evidence of a mutagenic or teratogenic effect of exposure to a foreign compound during pregnancy. This adverse effect is perhaps the most subtle and yet the most critical of all the potential problems that might develop from foreign chemical exposure. Not only are the observed defects first seen only months after the exposure and resulting cellular damage have occurred, but sterility of gametes, death of the fertilized but unborn organism, or abortion also may result. From a clinical standpoint, these latter three end-effects are largely undiagnosable, if indeed they are even observed.

Figure 1 diagramatically presents the potential effect of teratogenic agents on gametes and various stages of prenatal development. Agents affecting gametes commonly produce sterility, but if viable gametes result, no effects on the fertilized product are seen. Chemicals present during the blastocyst stage (the fertilized unimplanted organism) commonly produce death of the organism, or they produce no effect and a normal individual. The embryo stage (implantation has occurred and cell differentiation is in process) is most susceptible to chemical interferences causing major structural abnormalities or defects. Death is possible but less common than during the earlier periods. During the stage of the fetus (differentiation is largely complete and organ growth occurs), the incidence of death is low and major structural abnormalities do not occur at a significant level. Abnormalities in organ function can result from exposure to foreign chemicals during this stage, but the fetus is relatively insensitive to foreign compounds and a normal individual usually develops.

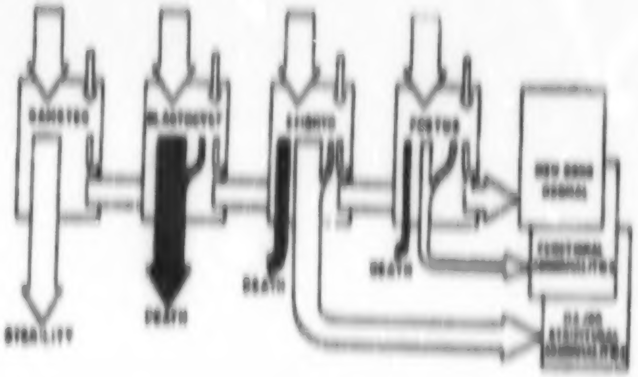


Figure 1.--Schematic representation of the influence of teratogenic factors on gametogenesis and various stages of prenatal development. Strong teratogenic agents--wide arrows; weak teratogenic agents--narrow arrows.

It is thus seen that the period of the embryo is the most critical stage for the unborn individual and that this is the period during which congenital defects or major structural abnormalities are most likely to result following exposure to teratogenic chemical factors. This particular period is crucial because of the sensitive condition of the differentiating cells of the growing organism. Each organ undergoes differentiation during a specific phase of this period; hence exposure to chemicals during the period of differentiation of the heart results in heart abnormalities, while exposure to chemicals during the period of oral cavity development results in defects of the oral cavity. The type of defect observed permits a judgment as to the time during which the teratogenic factor was present in the developing organism.

Table 6 summarizes the syndrome of mutagenesis and teratogenesis. The production of a structural or functional defect is the most common, but not the only, result. While a wide variety of causative agents may produce a defect, chemicals are one of the more important etiological factors, and they appear to be becoming more significant with the increased use of chemicals in everyday activities and their presence in the environment. Any one of the reproductive stages may be involved in this syndrome, either through direct effects upon the gamete or rapidly growing and dividing cells, through the production of toxic metabolites and their effects on growing cells, by altering nutrition to the growing organism, by producing variations in cell division and growth, or by the interactions and synergistic effects of several of the above factors. The subtleness of this problem and the difficulty in relating a cause to the end product not only makes this syndrome difficult to clinically interpret, but research and the documentation of causative factors are subject to much controversy. This problem will continue to be of major concern for many years.

TABLE 6

Mutagenesis (Teratogenesis)

---

1. Production of structural or functional congenital defect.
2. Wide range of causative agents.
3. May affect gamete, blastocyst, embryo or fetus.
   a. Direct effect
   b. Through metabolites
   c. By altered nutrition
   d. Through variations in cell division and growth
   e. By synergistic effects of several factors
4. Subtleness of effect causes much concern.

Sites of Chemical Action

The variety of chemicals that may cause toxicity, adverse effects, or participate in chemical interactions is abundant. Equal in variety are the biological sites at which these chemicals may produce their action. All produce their effect by reacting with one or more cellular components, and as illustrated in Figure 2, the "typical" cell has numerous foci at which chemicals may initiate a toxic or adverse effect.

The first cellular structure that a foreign chemical contacts is the cell membrane or wall. Here the chemical may cause coagulation of proteins or may inactivate vital enzymes responsible for maintaining the integrity of the cell. Protein precipitation or enzyme inactivation results in not only the cell losing its integrity, but also allows penetration of the foreign chemical into the innermost portions of the cell. Once inside the cell membrane, the chemical may react with several intracellular structures. The endoplasmic reticulum, containing ribosomes, is responsible for the metabolism of naturally-occurring and foreign compounds; proteins and enzymes synthesized by the ribosomes produce the catalytic activity for biotransformation. The presence of toxic amounts of a foreign chemical may result in altered protein and enzyme production and a decrease or cessation in the cell's capacity for biotransformation. The lysosome contains packets of enzymes responsible for digesting foreign material introduced into the cell. The digestive enzymes (lysozymes) are kept within the isolated packets until required by membranes

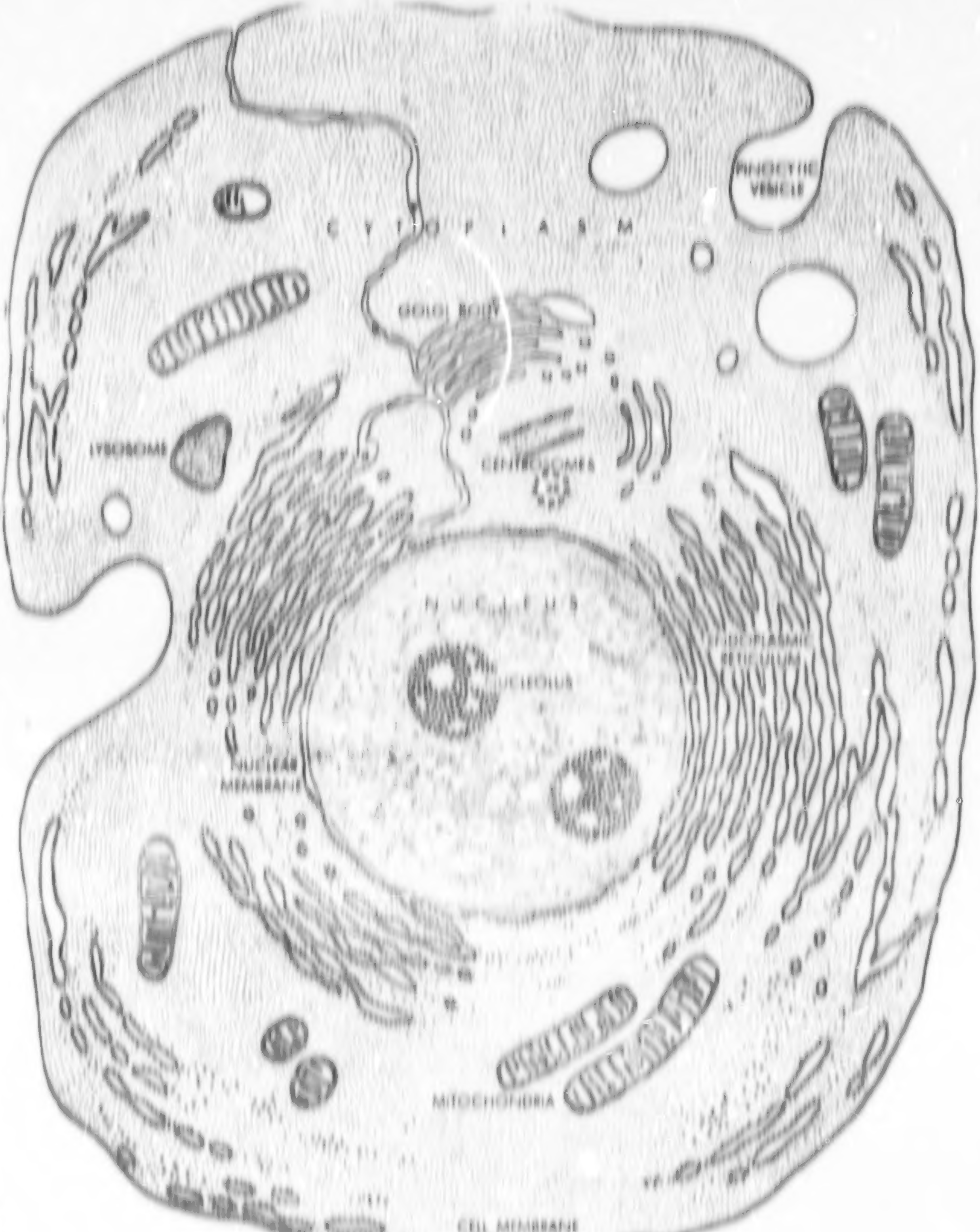

PINOCYTIC VESICLE
CYTOPLASM
GOLGI BODY
LYSOSOME
CENTROSOMES
NUCLEUS
ENDOPLASMIC RETICULUM
NUCLEOLUS
NUCLEAR MEMBRANE
MITOCHONDRIA
CELL MEMBRANE

Figure 2.--The schematic cell.

similar to those surrounding the cell. A foreign chemical which has already penetrated the cell membrane may also disrupt the integrity of the lysosome membrane and lead to release of the digestive enzymes into the cell is cytoplasm. Self-digestion or autolysis is the usual result.

The mitochondria are the generators of the cell and produce the energy required to keep cellular function at a normal level. The electron transport system, oxidative phosphorylation, and the production of ATP are all actively involved with the mitochondria. Foreign chemicals within the cell may enter the mitochondria and disrupt their energy-producing capability, resulting in "short-circuiting" the cell and power failure. A cell without energy cannot function and death is imminent. The nucleus is the central portion of the cell and contains the genetic material enabling reproduction and the ability to form normal daughter cells. Foreign chemicals entering the nucleus are capable of disrupting the genetic material (chromosomes), resulting in complete loss of reproductive ability or altered capacity to produce normal cells. In the latter instance, abnormal cells may develop and lead to the production of tumors, or in developing individuals, birth defects (teratogenesis) are the visible outcome.

In all instances, interaction with these cellular sites causes a disturbance of some vital portion of the cell, leading to altered cellular function and frequently death. The particular cell or cells affected vary with the route of chemical exposure and the specific sensitivity of the various body tissues to the particular chemical or chemicals introduced. The affected cells may be in the liver or kidney, or they may be red blood cells. Enzymes, in the brain or elsewhere, may be affected. On occasion, the protein of digestive tract cells may become severely coagulated and destruction of the intestinal tract lining becomes a prominent clinical effect. The specific affected cell and the resulting clinical signs, however, are largely determined by the characteristics of the chemical within the system. The basic effect is biochemical alteration of cellular function; the altered functioning results in physiological changes that are observed as signs and symptoms of toxicity.

Health Implications

The implications of the existence of one or more foreign chemicals within the body is thus apparent. Increased or decreased effects of therapeutic or environmental agents may result from the concurrent presence of other intentionally or accidentally introduced substances. The increased effect of a chemical due to interactions with other biologically present compounds may result in increased patient response and adverse effects or toxicities. Adverse effects, those reactions for which the clinician is unprepared since they are not expected, may produce life threatening emergencies because they are frequently unrecognized until clinical signs become severe. The onset of signs is frequently sudden, making effective treatment additionally difficult.

Chemical interactions resulting in a decreased effect of foreign chemicals, such as therapeutic agents introduced for a positive disease controlling response, may complicate and even nullify any useful purpose of the drug.

This unexpected lack of effectiveness in a therapeutic agent is usually unrecognized for several days, at which time the patient is noticed failing to respond or worsening. If these frank chemical interactions leading to acute effects are difficult to diagnose and compensate for, the problem of the more subtle interactions, resulting in immune situations, chemical resistance, tumor and cancer formation, or the after-the-fact observation of embryonal death and birth defects, can easily be seen to present much more complicated and confusing clinical problems. Their clinical recognition is only the first step. Understanding the mechanisms and specific chemical-biological interactions producing these consequences is a task that will require large scale dedicated and conscientious effort.

Even what little is currently known of the interactions involved in the "normal" processes of chemical metabolism and excretion is a vast amount of material (1-4,7-9). Figure 3 is a simplified illustration of some of the more significant interrelationships that occur in the biotransformation processes of any single given chemical. How much more confusing the scheme would be if all the known details of the interrelationships were included. How infinitely more complex the entire picture would be if we were to include the interactions possible when two or more foreign chemicals were present and competing for the same processes. Interested individuals are encouraged to consult the publications listed in the references for a review of the basic information currently available about chemical interactions (1-9).

Mechanisms of Chemical Interactions

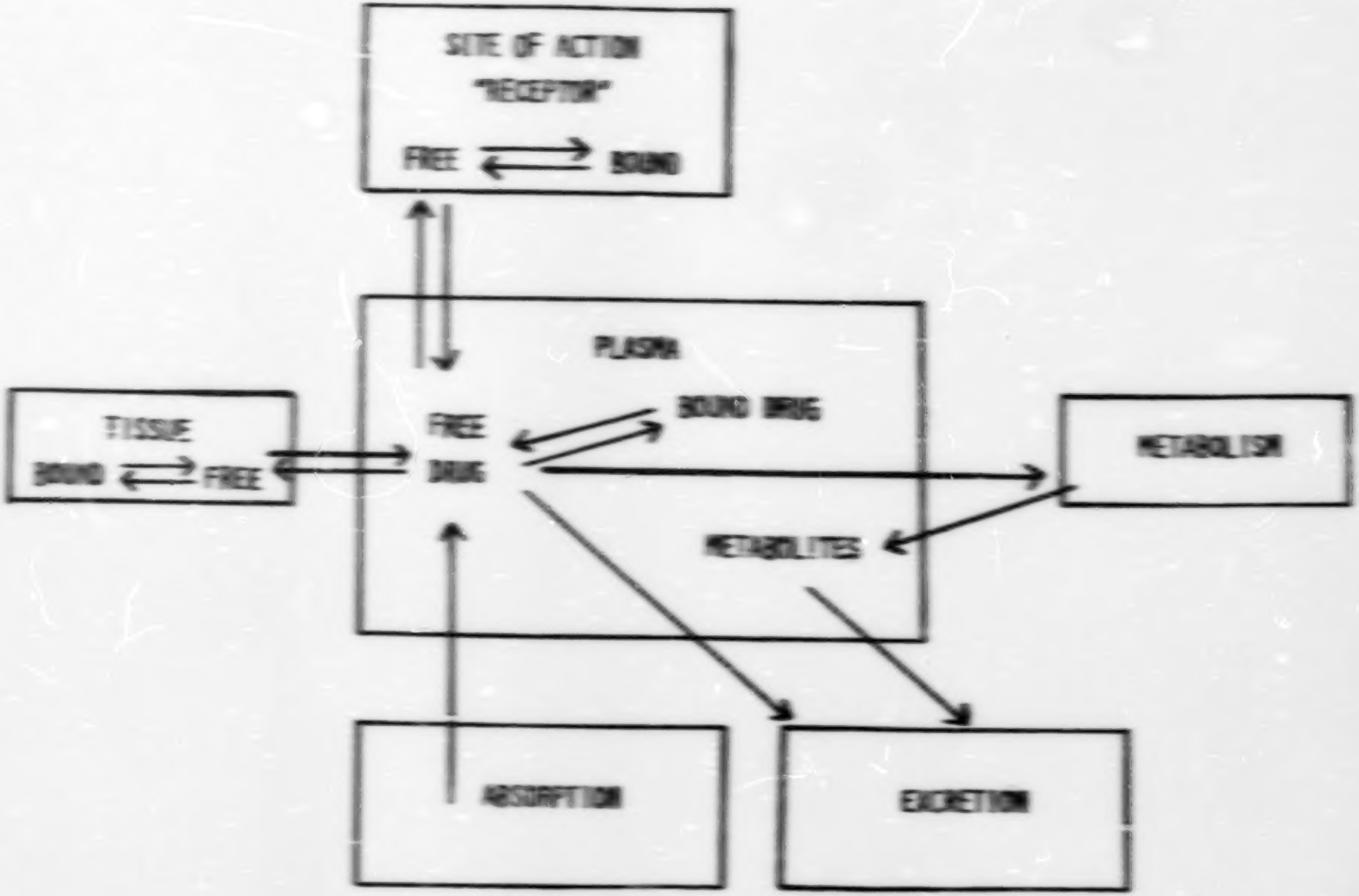
From the available literature, it is possible to speculate about the mechanisms that control and affect chemical interactions (3,8). The major types of such mechanisms are outlined in Table 7.

TABLE 7

Possible Mechanisms Affecting Chemical Interactions

1. Direct effect or chemical reaction between agents.
2. Modified biodynamics and detoxication.
   a. Altered intestinal absorption.
   b. Altered topical absorption.
   c. Unusual biological distribution.
   d. Modified action at receptor site.
   e. Altered biotransformation.
   f. Altered pattern of excretion.
3. Potentiation or inhibition.
4. Modifying physiological variables.
5. Interaction or environment.
6. Multiplicity of mechanisms.

Figure 3. Interrelationships between various biotransformation processes


SITE OF ACTION
"RECEPTOR"
FREE
BOUND
PLASMA
FREE
DRUG
BOUND DRUG
METABOLITES
TISSUE
BOUND
FREE
METABOLISM
ABSORPTION
EXCRETION

A direct effect of several chemicals on receptors or various cellular sites is the most obvious possible mechanism. Chemical reaction between two or more compounds present in the system, resulting the potentiation of effects or canceling of one or more of the chemical actions are also apparent direct actions that might occur. Much of the information available about these direct effects is the result of extrapolation of information about individual chemicals, their direct actions, and their interactions with cellular materials and other chemical compounds. Some of this information has been documented in patients; other aspects remain to be shown of clinical significance.

A very important and multifaceted potential mechanism for chemical interactions is the possible effect of several chemicals modifying the normal biodynamics and detoxification processes of the body. Variations in intestinal absorption may result from alterations in digestive tract pH, variations in intestinal motility and bacterial flora, chemical complex formation within the digestive tract that results in nonabsorbable salts and other inert compounds, complex formations that produce osmotic effects, complex formation resulting in sequestration or chemicals as lipoid materials or precipitates, alterations in the digestive tract mucosa, effects upon transport systems and mechanisms within the digestive tract, and circulatory alterations due to chemical interactions in the intestinal tract.

Similar alterations in absorption may occur if the chemicals are applied topically and the effects previously cited for the digestive tract occur in the mechanisms responsible for absorption through the skin.

Modifications in pharmacodynamics will exert their effect upon the normal pattern of foreign chemical distribution within the body. Transport systems, such as those active in the flow of physiological fluids, physical factors within membranes and transport systems, and specific transport across membranes may be altered. The presence of multichemicals within the system may result in displacement of one or more chemicals from binding sites or from storage tissues. Thus distribution alterations result in effects upon transport systems or upon storage or binding sites due to chemical-chemical or chemical-tissue-chemical interactions.

Additional modification may occur at receptor sites responsible for normal biological responses. Competitive inhibition, physiological antagonism, or nonequilibrium-noncompetitive antagonism may occur at cholinergic or adrenergic receptors. One or more of the foreign chemicals may act as partial agonists. These multi-actions can result in a variety of alterations from the expected receptor response.

When more than one foreign compound is present within the system, the biotransformation systems responsible for altering and detoxifying these foreign chemicals may become saturated or in other ways be forced to alter normal metabolic pathways. Enzyme inhibition and enzyme induction may effectively increase or decrease the duration of action and effect of a foreign compound. Other complex mechanisms, as yet undescribed, are also potentially possible.

The excretion of a foreign compound is usually a reliable means of ending that chemical's effect upon the body. Alteration in the pattern of excretion may markedly affect the length of time and effect that a foreign compound may exert upon the body. Multiple chemicals within the system may alter the urinary pH, have a direct effect upon the kidney tubules (thus changing expected absorption or excretion patterns within the tubule), or cause complex reactions involving more than mechanism to occur.

Chemicals reacting between themselves may cause potentiation or inhibition in the effects of one or more of the compounds present. Further, physiological factors that commonly would determine a chemical's influence upon the body may be altered by other chemicals being present at the same time. Factors such as age, body temperature, nutritional and pathological state, sex, species, and genetic make-up may all be modified by the presence of additional chemicals.

The interactions produced by environmental factors have not received great attention. From the nutritional standpoint, diet and influence of carbohydrates, proteins, minerals, vitamins, and water upon simultaneously ingested foreign chemicals is an obvious factor that has long been overlooked as influencing the effect of foreign chemicals. Even ambient temperatures and whether the subject is housed in a cold, normal, or hot relative environment may greatly influence body metabolism and activity; hence, it could play an important role on the effect of circulating foreign chemicals.

Like so many biological reactions, it is likely that no single mechanism or explanation will be ultimately found to be the only significant one. It is more plausible that a multiplicity of mechanisms, involving many or all of those heretofore theorized, are actually working to influence chemical interactions. The specific identification of these mechanisms, however, is currently a great goal for relevant and important investigations.

All health scientists await, and hopefully are "doing their thing" to contribute to, answers to these vital questions. Without documentation and understanding of how the presence of more than one chemical within the system will influence that system's functioning, man and animal are at the mercy of the therapeutic and environmental agents so frequently applied to patients and the general population. By understanding these interactions and the factors that govern them, health scientists and clinicians will be taking a giant step toward reducing the hazard from foreign chemicals.

An awareness of this increasingly common chemical syndrome is required to hasten the development of the capacity to treat and eventually prevent chemical interactions. It is hoped that this review will stimulate a few inquiring individuals to pursue this problem and to strengthen the painfully limited information currently available.

Summary

Chemical interactions are those biological responses not expected, but frequently observed, when two or more foreign chemicals are present in a biological system at the same time. As increasing numbers and varieties of foreign compounds are superimposed upon the body, it is understandable that undesired reactions are becoming more frequent. The presence of two or more compounds in the body may result in a decreased biological response, an increased biological response, or a new unexpected toxic reaction. These biological responses may be the result of receptor-chemical blockade or interaction, enzyme inhibition or induction, interference with enzyme systems, or cellular reactions resulting in altered organ function and chemical elimination. Since most interactions are not well-documented and even less understood, the clinician may suddenly be faced with an unexpected response to his patient. His reaction, whether to increase or decrease the administered dose, attempt antidotal therapy, or do nothing but observe and monitor vital signs, may be vital to the outcome of the case. Many clinicians accept such unexpected responses and discard their significance as an "individual variation." There is a definite need and responsibility to recognize, document, and attempt to understand these chemical interactions in order to prevent and control the unfortunately frequent life-threatening episodes that may result.

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EXPOSURE MEASUREMENT

Anne R. Yobs

Exposure measurement is not a new problem, nor is it restricted to chemical pollutants--although certainly the term has been extensively bruited about in connection with environmental considerations recently. The physician and public health personnel who work with infectious diseases have been measuring exposure since it was first realized that certain clinical entities are caused specifically by infectious agents and since laboratory procedures were developed for diagnosis of exposure to these agents. These include culture, microscopic examination of parasite or tissue, hematology and serology tests and certain measurements of physiologic change. As we will discuss in greater detail later, exposure to chemicals is usually measured with other procedures, although at times these same procedures may be used. The importance of accurate exposure measurement is pointed out by the following definition of epidemiology:

> Epidemiology has been defined as a science of mass phenomena. It describes significant varieties of the phenomena in time and place. It draws upon statistical methods and theory, all phases of medicine and the natural sciences to give a true picture of the occurrence, distribution, and types of defects. The proper use of this material, however, requires that it be carefully and accurately collected by reliable individuals who are able to describe what they observe. It must also be recorded in such a manner as to be recoverable when required alone, or to be synthesized with other material for future use.
>
> The objective of an epidemiologic study or investigation is to determine the circumstances that have led or will lead to selective exposure of certain individuals in a population to those factors--whether infectious, chemical or physical--that result in the development of an illness or abnormality. Successful study not only leads to understanding and identification of etiologic agents but should also reveal one or more alternatives for prevention. Thus, the epidemiologic approach is different from but related to clinical and biostatistical studies of disease and to preventive programs. Successful epidemiologic study requires the accurate identification of exposure and of cases to insure that cases in the group under study represent the same problem. In addition, characteristics of the case series may be compared with traits of an unaffected group or area where there is no problem and it is assumed there is little or no risk.

Let's restrict our attention to environmental pollutants and consider the value of exposure measurement information before discussing specific details of procedures required for its development. Exposure, that is contamination, may be measured in any environmental substrate. This data when combined with certain other information about production, usage, i.e., introduction into the environment, etc., makes it possible to define distribution and trace movement (of the material) through the parts of the environment as well as on a geographic basis. This mass of information is also necessary to the evaluation of environmental quality at a given time and changes over a time interval; this evaluation permits determination of the impact of certain regulatory decisions and enforcement actions or other occurrences. On an individual basis, this type information is useful in evaluating--and improving--working conditions in selected industries and in the diagnosis and treatment or prevention of acute or chronic poisoning.

Exposure measurement would be relatively easy to accomplish if available methodology permitted use of the same approach in investigating varying problems and pollutants and if the amounts involved in exposures were larger.

Exposure may be measured in several ways; however, no one way is applicable to all problem situations; each is suited to some selected situations and not to others. These methods may be divided into two groups: direct and indirect. Either may apply to individual or ambient measurements. Direct exposure measurement is the quantitation of the chemical or its metabolites contained in a sample of an environmental substrate such as air, water, soil, and food products. It may also be used to determine surface contamination of plants, animals, or man, or to estimate prior or current exposure by measuring the amount of a chemical, or its metabolites, which is present in the body or which is excreted from the body. Indirect exposure measurement requires quantitation of specific physiologic effects known to occur as a result of exposure to that chemical, such as enzyme induction or inhibition, change in nerve impulse transmission or cardiac rate, change in organ function, or induction of abnormal growth, etc. These changes must be specific for the chemical or chemicals under consideration.

Entirely different laboratory facilities may be required for the different methods and when the same method is applied to different chemicals. Direct exposure measurement and measurement of storage or excretion levels generally call for an analytical chemistry laboratory facility and competence, while measurement of physiologic effects may require an entirely different type laboratory facility. For instance, in studying exposure to the pesticide parathion, the parent chemical may be measured directly or urinary excretion of the metabolite para-nitrophenol or the alkyl phosphate metabolites may be measured; these all require an analytical chemistry laboratory with gas liquid chromatograph and flame photometric detector. On the other hand, if parathion exposure is measured in terms of cholinesterase inhibition, a biochemistry laboratory is needed; and if in terms of nerve impulse transmission, a neurology laboratory with electroencephalograph is required. Similarly, exposure to radioactive materials may be measured directly or indirectly and a pathology laboratory may be required instead of a scintillation counter laboratory.

In the field and in most research situations, it is not possible to use all available methods for exposure measurement nor to perform exhaustive studies to determine total exposure. That method must be chosen which is most appropriate to the problem by virtue of applicability, sensitivity, specificity, possible routes of exposure and restraints of available laboratory facility, staff competence and financial support. Routes of exposure vary with species and substrate, for example:

Man & animals	Ingestion	Respiration	Topical (dermal)
Fish	Ingestion		Topical
Soil			Topical
Plants	Root absorption		Topical
Water	Direct introduction		
Air	Direct introduction		

The significance of the route depends on the chemical, the dose, and the time interval of exposure.

Problems associated with exposure measurement will differ with the situation being investigated. These may be identified as follows:

- Ambient
- Individual specific
  - ambient
  - occupational
  - poisoning
- Research
- Field
  - incident
  - survey

Ambient studies may be directed to one or a wide range of pollutants. Generally, levels encountered in ambient surveys are quite low and their variety may be broad. This is true to a lesser degree in ambient studies of a specific individual or location. Occupational or poisoning studies may be somewhat less difficult because higher levels are involved and because it is frequently possible to identify the specific pollutant through field investigation before samples are sent to the laboratory. The availability of such information greatly simplifies the problems of sample collection and laboratory analysis.

Field studies may also be directed to one or a wide range of pollutants; this is especially true for field surveys but may also apply to incident investigations. In the latter case, levels of specific pollutants encountered are usually higher than in surveys, but it should be recalled that ambient levels of pollutants are present in addition to the suspect pollutant(s).

In research studies, it is possible to limit the scope of activity to a restricted area and to use a maximum amount of time in developing data. It should be noted that currently the measurement of man's total exposure to many pollutants would of necessity be a research project. It is entirely conceivable that in the future the further development of sensors will simplify this problem.

All exposure measurement studies require careful preplanning, as well as consultation with field and laboratory personnel on a variety of topics:

1. Determination of goals: Can they be recalled? Will this be a negative study? A false positive study? How many samples are needed; what frequency?
2. Location of sampling sites and establishment of sampling schedule: What sampling techniques will be used? Is it simple or sophisticated? Will it retain the pollutant of interest? Who will collect samples? Is training needed? What are the requirements for sample containers and sample transport?
3. Analysis: Is the identity of the material of interest known or suspected? Is methodology available for this material? Is it both sensitive and specific enough for the problem? Will the sampling technique have to be one that will concentrate the pollutant? Is the laboratory staff competent in the analytical procedure? Equipped? Is the material stable? What is the maximum allowable time between sample collection and analysis for data to be valid? How much confidence do you have in the laboratory--internal quality control, interlaboratory quality control (reference laboratory), instrument maintenance? Are suitable reference standards available? What interferences might be encountered? What confirmatory techniques will be used?
4. Data evaluation: Is data to be combined from other laboratories? Other studies? Other methodologies? Use extreme caution in interpretation. Who will evaluate data? How?

Failure to consider each of these points fully will jeopardize the success of the study.

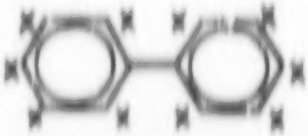
It is obviously impossible to speak to each of these points for all conceivable pollutants within the allotted time and space. To do so would be misleading in the future because of the rapid developments being made in environmental techniques. Therefore, it is recommended that thorough library research and consultation with individuals knowledgeable in the field be performed as the first step in studies involving exposure measurement.

In summary, exposure measurement is required for proper case or problem identification or for problem prevention. It is critical in toxicological matters where there are definite relationships between time and dose and the development of effects. Exposure may occur by several routes and may be measured in several ways; however, no one way is applicable to all situations, each being suited to some situations and not to others. Successful studies in the area require teamwork and careful planning at all stages.

PCB'S IN HUMANS

Anne R. Yobs

The polychlorinated biphenyls (PCB's) were described in the literature in 1881 (7), but they were not formulated and fully developed for industrial use until 1930. The PCB's (trade name Aroclors in the U.S.A., Phenoclor in France, Colphen in Germany, and Kanachlor in Japan) are manufactured in the U.S.A. primarily by Monsanto Chemical Company. The basic structure of PCB's is shown below where "x" represents any number of possible positions for chlorination leading to about 210 possible combinations, of which 102 are probable. Widmark, who made this calculation in developing criteria for these limitations, noted that the criteria were based on compounds containing five to eight chlorine atoms per molecule, the number of chlorine atoms per ring differing by not more than one (8).



The Aroclors may consist of chlorinated biphenyls, chlorinated terphenyls, or a mixture of these compounds. Invariably, although a specific numbered Aroclor may represent a molecular type and degree of chlorination or weight percent of chlorine, the product is in actuality a mixture of several of the compounds in a series. The first two digits represent the molecular type and the last two digits give the weight percent of chlorine. For example:

1200 = Chlorinated biphenyls
2500 = Blend of chlorinated biphenyls and
chlorinated terphenyls (75:25)
5400 = Chlorinated triphenyls

The Aroclors are chemically inert, making them ideally suited for certain industrial uses. They are not hydrolysed by water, and they resist alkalis, acid, and corrosive chemicals. Since they are not volatile, their boiling points range from 278°C to 451°C. They are stable to long heating and can be distilled at ordinary pressure without any carbonization or decomposition. As might be expected since they are insoluble in aqueous media, they are quite soluble in hydrocarbon solvents. It is believed that PCB's are more stable than DDT and metabolites and, accordingly, resistant to biodegradability or biological decomposition.

PCBs and formulations thereof have a wide range in viscosity and physical state. Other properties such as low solubility in water and high dielectric constants make them versatile for industrial uses. Many of these PCB preparations have strong adhesive properties. Aroclors could be used to extend the effectiveness or lethality of organochlorine insecticides such as chlordane, aldrin, and dieldrin in pesticidal formulations.

PCB's have been in use for more than four decades, not only in the United States but throughout the developed world. The physical and chemical properties that make polychlorinated compounds valuable industrially assure their ability to become serious persistent environmental pollution hazards. These properties include high boiling points, low water solubility, high dielectric constants, miscibility with organic solvents and polymers, remarkable thermal stability and resistance to chemical degradation. Industrial uses may be classified as "closed" or consumptive. Closed systems include electrical capacitors and transformers, heat transfer systems and hydraulic fluids. Consumptive uses involve synthetic rubber, paints, plastics, wire insulation, caulking material, flame retardants, cutting oils, pesticide synergists, adhesives, sealants, paper printing inks and protective coatings for wood, metal or concrete. Inadvertent environmental contamination can occur accidentally from closed systems, but environmental infiltration is inherent in the consumptive uses.

Data on sales of PCB's are available only for the United States from 1957 to 1971, with sales reaching a high of 36,000 tons in 1970. Sales doubled from 1960 to 1970; assuming the same growth rate from 1930 to 1970, about 300,000 tons have been sold in the United States. Data from outside the United States are few. It is estimated that Japan manufactured 13,000 tons per year (3). PCB's are also produced in West Germany, the United Kingdom, Spain, France, Italy, Russia, and possibly new producers in Brazil, Argentina, India, and East Germany. Assuming that the United States used half of the world total, world production would have been about one million tons--approximately half the estimated total production of DDT. Monsanto's 1971 sales dropped to half the 1970 level, and 1972 sales are expected to be 12 to 15,000 tons. Prior to 1971, when Monsanto (the sole U.S. manufacturer) curtailed sales to non-closed system uses, about 40 percent was used in plasticizers, hydraulic fluids and lubricants, surface coatings, inks, pesticide extenders, and microencapsulation of dyes for carbonless duplication paper--uses that potentially result in environmental contamination.

If the same percentages held worldwide, 40,000 tons might have been used in ways that could easily reach the environment; accidents and careless disposal practices would have increased this amount considerably, perhaps to 50,000 tons or more.

Nisbet and Sarofim (4) provided rough estimates of the losses of PCB's to the North American environment in 1970: 1500 to 2000 tons to the atmosphere (mostly Aroclor 1254 to 1260 from plastics and 1242 from burning dumps); 4000 to 5000 tons to fresh and coastal waters (Aroclor 1242 to 1260); 22,000 tons into dumps and landfills (mostly Aroclor 1242). Other losses were judged to be small, but often locally significant. The total loss to the North American environment from 1930 to 1970 was estimated to be:

Atmosphere	30,000 tons
Water - fresh and coastal	60,000 tons
Dumps and landfills	300,000 tons

The total of 390,000 tons is within a factor of two of the above estimate of 500,000 tons that might have reached the world environment. They further estimated that one-third of the PCB's released to the air and one-half of those released to water have now been degraded. The PCB's in dumps probably have undergone less degradation.

Given the diversity of uses of PCB's and their chemical stability (greater stability in the higher chlorine species), it is not surprising that residues are now widespread. While satisfying quantitative estimates of the contribution of various pathways into the environment are not possible with existing data, there are enough data to be certain that they do reach the environment at least from the following sources:

-- Open burning or incomplete incineration (at usual temperatures) of solid wastes, municipal and industrial. Incineration at 2000°F or above for two seconds will destroy PCB's, but poorly operated incinerators or open burning may result in PCB's being released unchanged to the atmosphere.

-- Vaporization from paints, coatings, plastics, etc. Nisbet and Sarofim (6) estimate that as much as 20 percent may be vaporized.

-- Municipal and some industrial sewers (present in treated as well as untreated wastes). See Tables 2 and 3.

-- Accidental spills or improper waste disposal practices.

-- Formerly, direct application to the environment as ingredients of pesticides or as carriers for pesticides (such uses are now prohibited).

-- Dumping of sewage sludge, municipal and industrial solid waste, and dredge spoil at sea.

-- Sewage sludges disposed of on land.

-- Migration from surface coatings (paints, etc.) and packaging materials into foods and feeds.

Probably the largest amounts of PCB's circulating in the environment reach it through industrial and municipal discharges to inland and coastal waters. They were not recognized as environmental contaminants until 1964-1966 when Jensen in Sweden identified a series of unknown peaks on gas chromatograms of pesticide analyses as these substances (4). These first identifications were in fish and bird tissues; examination of other samples soon revealed that PCB's were widespread in biological materials. Existing data suggest that although the greatest concentrations of residues are found in the vicinity of industrial and municipal areas in the Northern Hemisphere, residues exist in areas remote from civilization and in both the Northern and Southern Hemispheres.

In 1971, the PCB's were included routinely in the list of chemical residues for which every sample of human adipose tissue was routinely analyzed in the Human Monitoring Survey (8). In this study, which was initiated in 1967, samples are collected through the direct cooperation of pathologists who are in hospital or private practice or who are in public service as city or county medical examiners. Samples are collected from both sexes in all age and racial groups. Adipose samples are collected from tissues removed for therapeutic surgery or from postmortem examinations, the latter including people who have died accidentally (trauma) or during relatively brief hospitalization. No samples are accepted from institutions for long-term care of the chronically ill patient.

All samples are analyzed by laboratories established under contract in several states. These laboratories use analytical methodologies specified by the program and are required to maintain acceptable levels of performance, as demonstrated in both inter- and intra-laboratory quality control programs.

An analytical method was adopted which would separate PCB's from pesticides permitting accurate quantitation of pesticides and confirmation of PCB levels. This methodology is a further modification of the Mills, Olney, and Gaither procedure in which adipose tissue is subjected to extraction by petroleum ether, acetonitrile partitioning, and Florisil cleanup. A portion of the resulting 6% ethyl ether/petroleum ether eluate, in concentrate form, is treated with KOH to effectuate dehydrochlorination of DDT and DDD to their olefins, thus eliminating the problem of separating these pesticides from the PCB's. Oxidative treatment is then applied to convert any interfering DDE to p,p'-dichlorobenzophenone which has an Rf value different from the PCB's. The PCB's are then determined by thin layer chromatography.

Analytical results for 637 samples have been reported and are summarized in Table 1. Of the 637 samples reported, 198 (31.1%) contained measurable amounts of PCB's and 125 (19.6%) contained trace amounts, with the balance being negative. These samples were collected from 40 pathologists in 38 cities distributed over 18 States and the District of Columbia.

TABLE 1

INCIDENCE OF POLYCHLORINATED BIPHENYLS IN ADIPOSE
OF THE GENERAL POPULATION OF THE NATION

	Reported	
	No.	%
Negative	314	49.3
Trace (<1.0 ppm)	125	19.6
1 to 2 ppm	165	25.9
>2 ppm	33	5.2
TOTAL	637	100.0

Positive samples came from every hospital, city, and state sampled. It therefore appears that these materials are widely found in the population.

Details of distribution by sex and race, collection procedure, and diagnostic groups are summarized in Table 2. The difference between figures for the positive and negative groups does not appear to be significant, although no statistical evaluation has been performed because of the small numbers in this report.

TABLE 2

DESCRIPTIVE STATISTICS OF SAMPLES ANALYZED

	Negative and Trace <1.0 ppm		>1.0 ppm	
	Number	Percent	Number	Percent
Sex				
Male	221	50.3	127	64.1
Female	218	49.7	71	35.9
Race				
White	381	86.8	151	76.3
Nonwhite	58	13.2	47	23.7
Samples from				
Therapeutic surgery	95	21.6	29	14.6
Postmortem	344	78.4	169	85.4
Diagnostic group				
Neoplasms 140-209	77	17.5	39	19.7
Circulatory system 390-458	121	27.6	61	30.8
Gastrointestinal system 530-565	44	10.0	16	8.1
Liver and gall bladder 570-575	20	4.6	6	3.0
Pancreas 577	0	0.0	2	1.0
Urinary system 580-595	11	2.5	7	3.5
Congenital anomalies 740-759	5	1.1	1	0.5
Accidents & violence E numbers	16	3.6	7	3.5
All other	145	33.0	59	29.8
TOTAL	439	99.9	198	99.9

Finklea et al. have reported results of a 1968 study of residents of Charleston County, South Carolina, distributed by race and residence. Their findings are summarized in Table 3 (1). PCB residues were found in parts per billion (ppb) concentrations in plasma. The prevalence of PCB residues varied remarkably over race-residence groupings being greatest in urban residents and rural whites. No clear independent age and sex trends could be demonstrated. PCB residues were not linked to chlorinated hydrocarbon residues commonly found in food and were only weakly linked to pesticide residues that might reflect recent household or garden pesticide exposure.

TABLE 3

PCB RESIDUES IN PLASMA (PPB)

	Number of Observations	Range	Arithmetic Mean
Rural Black	107	0.0 - 20.6	0.3
Urban Black	151	0.0 - 29.0	1.9
Urban White	166	0.0 - 23.0	2.3
Rural White	192	0.0 - 16.6	3.1

Because of their widespread industrial applications, their chemical stability and persistence, and their ubiquitous presence in the ecosystem, it is not unexpected that PCB residues have been detected in a variety of food commodities. Several sources of contamination have been identified. These can be generally divided into three categories:

1. Environmental contamination--background levels of PCB's in fish from contaminated lakes and streams.
2. Industrial accidents--isolated incidents involving direct leakage and spillage or contact of PCB fluids and other PCB-containing materials on animal feeds, feed ingredients or food.
3. Food packaging materials--PCB migration to foods packaged in PCB-contaminated paper products. Identification of these categories is not intended to imply that all positive PCB findings in food and other materials have been successfully traced to any one of the three sources. Samples have been reported where the cause of the PCB residue was not clearly defined, and one could only speculate as to the source of the residue.

Since November 1969, FDA has analyzed for PCB residues all raw agricultural commodities and other food classes sampled under its pesticide surveillance program. More than 13,000 sample examinations had been completed as of June 1971. A total of 279 of the objective samples was reported to contain PCB's. An additional 200 food samples, collected as follow-up samples of suspected lots because previous analyses indicated a potential problem, were also found to contain PCB residues. PCB's were encountered most frequently in fish, with 317 of the total positive samples (479).

Compared to the chlorinated hydrocarbon pesticides, definitive aspects of acute, sub-acute, and chronic toxicity still remain rather poorly known. Chloracnegen effects were reported as early as 1936, following industrial exposure to the PCB's. Occupational chloracne, however, has not been a problem with recent usage of the PCB's. Approximately ten cases of fatal intoxication involving persons who handled or were exposed to chlorinated biphenyls or naphthalenes in their occupations have been described. In all cases, histological examination revealed liver fatty degeneration, necroses and cirrhosis. It is important to note that chlorinated naphthalene as well as chlorinated dibenzofurans have recently been identified in two commercial polychlorinated biphenyl samples (Phenoclor DP6 and Clophen A60) (2).

Human intoxication with the heat exchanger Kanachlor 400, a Japanese manufactured PCB with 48 percent chlorine and containing as its main components a tetrachlorobiphenyl and a pentachlorobiphenyl, was reported in Western Japan in 1968.

More than 600 people were affected following the consumption of contaminated rice oil containing levels estimated to range from 2,000 to 3,000 parts per million PCB's (average of about 2,000 parts per million). Exposure levels to the oil were calculated to approximate 15,000 milligrams per day. The lowest reported figures allow an estimate of a minimal positive effect level at 3 milligrams per day over several months. However, the average doses associated with significant disease in the "Yusho" incident were much higher and were in the range of 30 milligrams per day (5).

The clinical aspects associated with "Yusho" included: chloracne, blindness, systemic gastrointestinal symptoms with jaundice, edema and abdominal pain. Chloracne is very persistent with some patients showing evidence of it after three years. Newborn infants born from poisoned mothers showed skin discoloration due to transplacental passage of PCB's. This skin discoloration regressed after 2 to 5 months. Gingival hyperplasia with pigmentation occurred in several cases. Decreased birth weights were also noted but no evidence could be obtained in regard to the possible retardation in physical and mental activities of the babies. The skin of stillborn infants showed hyperkeratosis and atrophy of the epidermis and cystic dilation of the hair follicles. Residues of PCB were found in fetal tissue.

Components of Kanechlor 400 with longer retention times were detected in sputa and fatty tissues of patients. Serum triglyceride levels were higher than 300 mg/ml in 60 out of 396 subjects investigated before the end of 1969. Among the incidence of hyperglyceridema (triglyceride >300 mg/ml) of the six decade groups (age 0-9, 10-19, 20-29, 30-39, 40-49, 50-), that of the first decade group was the highest while that of the third decade was the lowest. The hyperglyceridema was considered to be possibly due to decreased post-heparin lipolytic activity and impaired plasma triglyceride removal. Cholesterol and phospholipid concentrations were increased while lecithincholesterol acyl transferase activity was decreased. In female patients, plasma lipoprotein lipase activity was decreased, and steroid secretion was elevated.

Autopsy tissues of two Yusho fatalities contained chlorobiphenyls in all organs examined, especially mesenterial fatty tissues, skin and bone marrow. It was assumed that their presence might have been responsible for the observed long duration of the intoxication symptoms (2).

In summary, PCB's have been shown to occur at low levels in the general population of the United States. The source of exposure to these chemicals has not been finally identified. Levels are far below those reported in an epidemic poisoning which occurred in Japan.

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EPIDEMIOLOGY OF POISONING BY CHEMICALS*

Frank S. Lisella

During the pre-World War II era, the hazards associated with the use of many common chemicals went unheeded by the nations of the world. In 1940, a British article was published implicating aspirin in 43 suicides, 8 accidental deaths, and 14 unclassified fatalities in Great Britain during that year alone (2).

A series of accidental deaths from acetylsalicylic acid were reported from the United States in 1950 (27). At that time, investigations revealed that from 1926 to 1943 a total of 227 persons had died from the consumption of excessive amounts of this chemical. These deaths were associated with the increase in production of just one of the salicylates--acetylsalicylic acid--which had exceeded 9 million pounds in one year.

Medicants of various kinds have been shown to play a significant role in childhood accidents and deaths. For example, oil of wintergreen was responsible for six deaths among children in England from 1931 to 1939, and 17 from 1940 to 1949 (30). The same chemical accounted for 28 deaths among children in the United States in 1951, and 34 in 1952 (13). Other products of concern have been broadly classified into categories such as disinfectants, anti-septics, household poisons, poisonous plants, and "miscellaneous."

A review of the accidental poisoning problem in the United States (5), prompted by the findings of British investigator Douglas Swinscow (30), revealed a total of 3,659 fatalities among children during the period 1940 to 1950. This represented a mortality rate four times greater than that among children in England during the same period, which was attributed, initially, to racial differences between the two populations. Medicants (drugs) accounted for approximately two-thirds of the cases in England, but about one-third of the cases in the United States. In the United States during the period 1949 to 1950, aspirin and the salicylates caused a total of 113 deaths in children under 5 years of age; barbiturates ranked second and accounted for 31 deaths. Iron poisoning, common in England, is rare in this country, and only two cases were found in this review. In one year, more than 100 deaths in the less than 5-year-old group were due to ingestion of petroleum products, one of the major factors responsible for increasing the rate in the United States over that in England.

In 1953, a Scottish researcher reported on a detailed investigation of poisoning episodes in that country (20) which showed that the number of accidental poisonings seen at the hospitals in Edinburg had risen from five cases in 1931 to 50 cases annually by 1951. This stimulated an investigation into the accidental poisonings seen at the hospitals in Edinburgh and Aberdeen, and the author reviewed 502 cases that occurred during that period. The principal agents responsible for illness among children in that study population are as follows:

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Agent	No. of Cases
Disinfectants	39
Kerosene	31
Turpentine	25
Bleach	22
Barbiturates	36
Iron Preparations	24
Salicylates	20
Camphor	29
Laburnum	15

Concern about the problem of accidental poisonings in the United States prompted creation of Poison Control Centers (15, 25). The first center was established in Chicago in the latter part of 1953 and operated on a city-wide basis. This center combined the efforts of the Departments of Pediatrics of all five medical colleges in Chicago, the Chicago Board of Health, the State Toxicological Laboratories, the Division of Services for Children of the University of Illinois, and the six major teaching hospitals in Chicago. An advisory committee that included representatives of the American Medical Association, the National Safety Council, and the Food and Drug Administration was formed. Each participating hospital was provided with a summary of references on the basic toxic constituents of the thousands of materials used in average households. Current knowledge on treatment for ingestion of these materials was also made available to each of the participating hospitals. A pediatrician was designated as Poison Control Officer, responsible for liaison between the central committee and participating groups, assuring the availability of necessary equipment and supplies in emergency rooms, orienting staff members to the problems of poisoning, and reporting to the Chicago Board of Health.

By March, 1958, 124 centers were in operation in 40 states (15), and this was increased to 380 centers in 49 states in 1960 (3). The general purpose of the centers is to minimize the danger of toxic substances by improving and expanding efforts for the prevention and treatment of poisoning.

In 1957, the Public Health Service reacted to the chemical poisoning problem by establishing the National Clearinghouse for Poison Control Centers (25). This group maintains detailed lists of the ingredients of all household products and drugs and provides the information to the local centers. Conversely, the local centers provide reports to the Clearinghouse, on any new poison hazards they discover for dissemination to all other centers. The Clearinghouse conducts research activities, investigates and analyses poison cases, and provides information on these and other activities to the local centers through a monthly newsletter. The Poison Control Centers provide the framework for detailed studies of the chemical poisonings in many communities throughout the United States.

Scientific articles published since the Poison Control Center network began include several dealing with epidemiologic analysis of case reports submitted to the National Clearinghouse (11, 12) and others reporting specifically on the chemical poisoning problem in selected metropolitan areas such as Chicago (43), New York (33, 34, 35), Salt Lake City (38), and Syracuse (52, 53). One of these reports (12) was an analysis of 3,926 cases of accidental poisoning. The age group of 5 and under accounted for 96 percent of the cases occurring in children under age 15 and 86 percent of the total. Males were reported to be involved in 53 percent of the total cases. Medicinal agents (aspirin, barbiturates, non-barbiturate sedatives, tranquillizers) were the offenders in 2,152 cases (54.8 percent). Household preparations such as soaps, detergents, and bleach accounted for 561 ingestions (14.3 percent), followed by pesticides, 315 cases (8 percent); the remainder (22.9 percent) were attributed to a variety of other products.

A paper by Howard H. Gann and colleagues reviewed in detail the results of 15,094 individual case reports submitted to the Clearinghouse (11). A similar pattern of age distribution was shown, with approximately 90 percent of the cases occurring in children less than 5 years of age. Internal medicants were ingested in 41 percent of the accidents, and aspirin was reported in over 50 percent of these cases. Where the type of aspirin was known, the "baby" type accounted for 84 percent of this total. Household cleaning and polishing agents were ingested in 17 percent of all the accidents. Ten percent of all the reported ingestions involved pesticides--the majority of which were insecticides. This article differed from those previously published in that an attempt was made to determine the type of container in which the ingested substances were found at the time of the accident. In one-third of the total cases, the chemicals were not found in the original container. Forty percent of the poisoning accidents occurred in the kitchen, 22 percent in the bedroom, and 12 percent in the bathroom; the remaining incidents occurred in the basement, living room, garage, and other areas.

The report on the study in Chicago (43) showed the results of analysis of 1,033 cases of poisoning reported from 19 hospitals between December 1953 and May 1955. A pattern characteristic of that shown earlier was found. Medicines were the primary agent, accounting for 523 (50.6 percent) of the incidents; of these, 210 were attributed to aspirin, and 196 cases involved cleaning, polishing, and sanitizing agents, with bleach the most frequently ingested chemical in this category. Poisoning with pesticides occurred in 107 (10.4 percent) of the total cases; insecticides were the primary agent in this classification, followed by rodenticides. There were six accidental deaths in children under 10 years of age, and six fatalities in persons 22 to 48 years of age which could have been either accidental or suicidal. In the majority of poisoning cases, the offending agents had been stored in the kitchen; in the remaining cases, they had been kept in the bedroom, bathroom, and other locations, in that order of priority. A predominance of the cases occurred among persons residing in low socio-economic areas; however, the author admitted that the possible bias in any conclusion drawn from this fact would be appreciable.

Chemical poisonings, excluding those due to gas, are the third leading cause of death in children under 6 years of age in New York City (32, 34, 35). In 1954, 454 cases of chemical poisoning occurred in children under 16 years of age in that city. Drugs were responsible for 47 percent of all poisonings, with aspirin as the principal agent and barbiturates as the second leading cause in this category. Bleaches were the primary agent in the household preparations class. Thirteen deaths were reported among children as the result of ingestion of lead-based paints; this finding corroborates similar investigations of lead-paint poisoning episodes in other areas of the United States such as Philadelphia (31), Baltimore (45), St. Louis (47), Cleveland (49), and Chicago (17).

A 1955 study confirmed the problem of poisonings in children in New York City (34). Twenty-one attempted suicides with chemicals were reported in children ranging from 11 to 16 years of age. Eighteen were girls and three boys. Age 13 was a high-risk category, accounting for nine incidents. Aspirin was responsible for 10 poisonings, followed by barbiturates, iodine, bleach, tranquilizers, antiseptics, and other agents. While no fatalities were reported among this group, seven fatal cases were reported in children ranging in age from 10 to 24 months.

A substantial increase in the number of poisoning incidents in New York City between 1960 and 1961 (35) was attributed to improved reporting by physicians. Sixty-six fatalities from poisonings occurred during this period, with barbiturates as the chief cause. Ten fatalities from barbiturates, mainly suicides, were reported in the adult group. Six fatalities occurred in children 5 years old, four from lead poisoning, one from talcum powder ingestion, and one from cough syrup. The reported number of cases rose from 5,070 for a six-month period in 1960 to 6,414 for the same time interval in 1961.

The study in Salt Lake City, Utah, in 1953 and 1954 revealed that 479 persons were treated for chemical poisoning in those years (38). Of this number, 311 were children and 168 adults. Drugs were by far the most common cause of poisoning in both children and adults. Aspirin accounted for 27 percent of all the cases in children. Four persons died from ingestion of chemicals--one child from cyanide and three adults from cyanide, strychnine, and alcohol.

An attempt was made to study the prevalence and characteristics of accidental poisoning in an urban population in Syracuse, N.Y. (52, 55) The authors obtained their data by direct interviews with a 2 percent sample of the total population of this city. Thus, 1,069 families with 4,069 members were selected. This group was considered to be reasonable representative of the total city population in age, sex, and socioeconomic status.

A total of 107 poisoning episodes were recorded among the study population. Medicines accounted for 63 of the poisonings, 33 of which involved aspirin. The remaining ingestions - 44 - were due to household products, petroleum-based chemicals, pesticides, paint and paint products, cosmetics, and other agents. Physicians were consulted in only 79 of the total cases. Some of the persons interviewed were not aware of the hazards of aspirin, and only about 66 percent of these ingestions were brought to the attention of a physician. This contrasted with the experience in other types of poisonings where 80 percent or more had sought medical assistance. The authors were not able to demonstrate any geographic of socioeconomic aggregation of cases.

M. A. Heasman presented morbidity data from England and Wales for 1958 and mortality data for the period 1954-1958 (29). The 1958 morbidity data were obtained from a 10 percent sample of all patients discharged from the National Health Service hospitals (excluding mental hospitals). From these records, 1,000 consecutive cases of poisoning were extracted. A total of 218 of these cases occurred in children under 15 years of age, about 50 percent of which involved the ingestion of pills, tablets, and other medicinal substances. In this category, aspirin accounted for 44 of the cases; barbiturates, 28; and other substances, 33. Turpentine, paraffin, and disinfectants ranked highest among the household products group, with 18, 16, and 7 cases, respectively. Berries and plant substances were responsible for 8 cases. Although there were no deaths among these cases, fatalities in this age group in England and Wales during the period 1954-1958 averaged 44 per year. As with the hospital admissions, aspirin and salicylates were the most common causes of death. Iron preparations were responsible for 12 deaths, and 10 deaths were attributed to strychnine.

Attention has been focused on the role of pesticides as causative agents in poisonings (10, 18, 22, 28). The extent of injury from pesticides on a nationwide scale is difficult to determine, but California publishes a detailed tabulation of nonfatal injuries from economic poisons annually (18). The difficulty in compiling reliable pesticide poisoning statistics has been due to the lack of uniformity in identifying and recording agents responsible for injury. For instance, from a total of 446 entries listed under the category of poisoning in the International Statistical Classification of Diseases, Injuries and Causes of Death, only 17 items relate to pesticides. There are, however, over 400 basic pesticide chemicals and approximately 45,000 formulations now available. In a study of pesticide mortality, it was shown that 196 deaths were reported in 1946, 154 in 1949, 152 in 1950, 132 in 1952, 137 in 1953, 105 in 1954, and 140 in 1955 (18).

Data submitted by various reporting groups such as industrial commissions and Poison Control Centers support the conclusion that only the more familiar materials used as pesticides are included in this category. A later report (10) included data on mortality from pesticides for the years 1956 to 1960. Pesticides were reported as the cause of death in 147 cases in 1956, 111 cases in 1958, 130 cases in 1959, and 103 cases in 1960. A paper published in 1964 (28) speculated that the rate of accidental deaths from pesticides was about one per million persons each year. It was further indicated that there had been no significant change in the death rate associated with the increased use of the new pesticides.

Results of detailed investigations conducted in certain high pesticide-use areas of the United States, however, showed a higher death rate. For example, in Dade County, Florida, which has a population of 1,040,000, 89 deaths due to pesticide ingestions were reported from 1956 to 1964. This statistic included 33 cases designated as accidental, 52 suicides, and 4 homicides. Parathion, an insecticide not specifically listed in earlier papers, was shown to be responsible for 9 of 10 deaths among children over a four-year period (22). A unique problem in Dade County is the illegal door-to-door sale of parathion in unmarked containers. This activity in itself has been shown to be responsible for the deaths of several children. Data on total poisonings from pesticides reported in three recent years are shown in Table 1.

TABLE 1

REPORTED POISONINGS BY PESTICIDES--UNITED STATES* 1968-1970

Pesticide	Cases			Deaths		
	1968	1969	1970	1968	1969	1970
Insecticides	2,919	2,929	2,913	11	14	13
Rodenticides	1,185	1,192	1,132	4	6	6
Fungicides	99	90	106	0	0	0
Herbicides	254	281	287	9	6	1
Mothballs	922	813	933	0	0	1
Miscellaneous	360	397	358	0	0	0
TOTAL	5,739	5,702	5,729	24	26	21

The etiology of accidental poisoning episodes has been the subject of considerable investigation. In addition to the more common safety hazards from improper storage of chemicals, other facets of the problem have been explored; for example, the role of oral abnormalities. J. O. Craig (21) showed that children who poison themselves can be divided into two groups, those who take the poison (usually in the form of attractive tablets or liquid), but who only taste or chew the substances and, in the majority of cases, reject them before harm results; and those who show exaggerated oral traits, amounting to pica in about 30 percent of the cases, and poison themselves with tablets of any kind. Tablets attract these children more than liquid and candy-flavored tablets such as aspirin or ferrous sulphate may be ingested in lethal quantities. The risk of death in children in this group is greater than in the first group.

An attempt was made by Australian workers in 1956 to correlate social factors with accidental poisonings among children (1). Results of their study indicated that negligence or carelessness by parents was an uncommon cause of poisoning. In follow-up investigations, the authors found that 70 percent of the poisoned children came from homes whose parents were conscientious in caring for their children; 63 percent of the 155 cases were from "standard" homes, and 68 percent of the fathers were professional people, businessmen or skilled tradesmen. The smaller number of children came from poorer families, belonging to the laboring or semi-skilled groups who had a lower material standard of living.

In other attempts to establish possible social factors in accidental poisonings, investigations were made of repetitive poisoning cases. One of these investigations (53) was based on the study of a group of 100 children who had experienced one episode of accidental poisoning. A group of matched controls was selected from a previous randomized population derived from the city of Syracuse (55). The poisoned group was found to experience many more subsequent episodes of poisoning than the control group. These children were more active and mischievous, were more likely to experience falls, and were more frequently separated from their parents. The girls in the repeater group were described as being more boyish, and both sexes walked earlier than members of the control group.

In a later study of the families of 20 poison repeaters, 19 single ingesters, and 13 controls, no correlation was apparent between repeated episodes of poisoning in children, and religious affiliation, income, geographic setting, or family size (48). Contrary to the study reported by Wehrle *et al.* (53), these investigations showed no relationship between repetitive poisonings and accident proneness, pica, environmental hazards, or lack of parental supervision. The ingestion of poisons seemed to be the result of purposeful behavior on the part of the child. Possible causes of the first ingestion were considered to be the result of the child's negativism, imitation of the parent's pill taking, or confusion with food. Subsequent ingestions were described as methods to get attention.

Baltimore and Meyer (6) suggested that improper storage and parental ignorance of toxicity were secondary environmental factors in accidental poisonings, although no statistical difference in poisoning incidents could be proved. A statistically significant relationship was found, however, between "pica" and "daredevilness" in children and poisoning accidents.

Scientific literature contains a variety of published reports on methods and programs for the prevention of accidental poisonings. The most successful program for reducing child deaths was the network of Poison Control Centers. Their influence on accidents is more difficult to determine since the Centers are not located in all urban and rural areas and are operated on a voluntary-reporting basis.

Other proposals for the prevention of poisoning incidents have related to labeling of containers for chemicals (19, 39). The need for precautionary labeling of chemical products was first formally expressed by the American Medical Association in 1884 with the adoption of a resolution urging Congress and state legislatures to enact laws requiring containers of lye to bear a poison label (19). Specific warning statements are required on various types of products under the Federal Food, Drug, and Cosmetic Act, the Caustic Poison Act, and the Federal Insecticide, Fungicide, and Rodenticide Act (18). The influence of labeling in decreasing accidental poisoning among children is difficult to assess, however.

A study, made to determine the degree of "attractiveness" of certain medicants, particularly aspirin, to children, indicated that bright colored tablets were the most popular, with magenta ranking first (37). Brown placed high among the study population, presumably because of its similarity to chocolate. Wine- and black-colored tablets were low on the list; therefore, the researcher proposed that aspirin and other drugs be incorporated in black-colored tablets and that tablets be dispensed in plastic strips which are difficult for children to tear.

In a study reported in 1959 (6), the use of safety closure caps on drug containers was proposed. Fourteen different types of safety caps were investigated over a period of three years, and two caps were finally selected for comparison with the conventional screw cap in a test area of 1,600 homes that had children under 5 years of age. Over 93 percent of the children in the survey had difficulty in removing the two experimental caps, and it was concluded that a safety cap was an important and practical safety measure. In a study in the Tacoma, Washington area (46) the author found that, after the introduction of bottle closure devices, the rate of accidental poisonings from tablets dropped from one per 5,100 prescriptions to one per 62,300 prescriptions. Poisonings due to children's flavored aspirin were reduced by 97 percent. Similar results were reported from a Canadian investigation.

In a detailed study of the etiology of a group of 100 poisoning incidents, Jensen and Wilson (36) found three circumstances, singly or in combination, that were common to 86 of the cases. In 26 cases, a person other than the parents (siblings, other children, neighbors, relatives, friends) played an important part in the episode. These individuals either made it possible for the poisoned child to reach the substance or failed to properly store the chemical. In 31 cases the parents were not aware that the child could climb, open doors, unscrew caps, or perform other tasks necessary to obtain the agent. In 53 cases, the poison was not in its usual location, either because it was in use, because persons other than the parents had left it out, or because the parents themselves were careless.

Studies have shown that many accidents could be avoided if parents would refrain from taking medicines in the presence of their children (9). A detailed study in the Norwalk, Connecticut area supported the findings that children will imitate their parents in this respect. It was shown that of the children poisoned by medication other than aspirin, 67 percent frequently observed their parents taking pills. This was found in only 13 percent of a comparison group who ingested "baby" aspirin. The investigator suggested that this factor in poisoning incidents could be minimized by proper parent education (40). Application of the traditional approaches to public health education has been suggested to reduce accidents from chemicals (34). Included among the educational techniques which have been employed are films, exhibits, lectures, demonstrations, and the establishment of poison prevention programs with community health councils, Parent-Teacher Associations, 4-H Clubs, and similar organizations.

A study conducted in Charleston, South Carolina, in 1962 and 1965 (42), demonstrated the practicality of a concentrated, community-wide program to reduce the incidence and severity of accidental poisonings. This survey pointed out that lack of awareness by adults still exists at all socioeconomic levels. One of the newest approaches to poison prevention is Poison Central (41) established in Kentucky in 1962, which provides a master toxicology information and service facility for the state system of regional poison information and treatment centers. Poison Central operates on a 24-hour basis seven days a week and provides toxicologic services not available at regional centers or at local hospitals and clinics. It also provides readily available services for areas not served by Poison Control Centers.

The deliberate ingestion of chemical agents or the so-called "therapeutic misadventures" or self-poisoning episodes, with or without suicidal intent, is a problem of great concern. This problem, particularly among young individuals, was noted in New York City after the Poison Control Center was set up in 1955 (33). The author of a paper on this subject in the New York area indicated that 299 suicidal attempts by poisoning were reported for individuals under 20 years old. These episodes took place between March, 1955, and December, 1958. Females accounted for 232 cases or 78 percent of the total. Puerto Ricans, who compose 11.8 percent of New York City's population, accounted for 28 percent of the attempted suicides. The primary agents involved were aspirin (84 cases--28 percent), barbiturates (47 cases--16 percent) and tranquilizers (36 cases--12 percent); the remaining cases were attributed to bleach, iodine, lye, insecticides, and other chemicals. A definite seasonal pattern was noted, with the incidence lowest in autumn and highest in spring. The type of agents used in the New York City cases have been implicated in similar episodes elsewhere (14).

James Toolan speculated on some of the predisposing causes to suicide (51). In his report, he discussed two cases involving the ingestion of chemicals. One of these was a 14-year-old male who swallowed an unidentified toxic agent to prove that he was invulnerable. Another was a 12-year-old girl who attempted suicide by taking a large amount of sedatives, because she dreamed of her dead mother and wanted to join her.

The hazards to health from the availability of proprietary drugs like scopolamine have been a matter of concern because of the involvement of these chemicals in self-poisoning incidents. This chemical has been the subject of many reports, including one from Casper, Wyoming (7) where six cases involving suicidal ingestions were treated in a six-month period. The authors of that report take exception to advertisements that emphasize the "safe" or "harmless" character of sedatives containing this chemical. Other investigators (16) have held similar views concerning this agent. The toxic dose is reported to be about 3 to 5 mg (12 to 20 tablets).

From the experience of these researchers, self-induced drug intoxications usually involve five main classes of agents--barbiturates, salicylates, glutethimide, meprobamate, and phenothiazines. Their experience also indicated that ethylene glycol, bromides, and scopolamine preparations are frequently ingested with suicidal intent.

A study dealing with the various aspects of self-poisoning in the Chicago-Cook County area showed that in 1964, 468 persons were treated for deliberate poisonings in the adult emergency department at Cook County Hospital (30). The preponderance of persons in the group were females (333--71 percent). Persons less than 45 years of age made up 84 percent of the group; more than half of these were less than 25 years old and a large number were teenagers. The patients ingested 102 different drugs and chemicals. Barbiturates were again the agent of choice, followed by the salicylates and tranquilizers. Rubbing alcohol, bleach, antiseptics, and rodenticides were also used as self-destructive agents.

Two comprehensive studies on self-poisoning episodes have been published by the British in recent years (23, 26). In the Oxford area, it was shown that 503 females and 200 males, ranging in age from 11 to 85 years, were admitted for treatment of self-induced poisonings between 1962 and 1965. Females outnumbered males in all age groups. The authors reported that there were 40 double admissions, seven triple, two quadruple, and one sextuple. The main poisons used were barbiturates (65 percent of the cases) and aspirin (26 percent). The use of barbiturates rose steadily with age from 23 percent in the age group under 20 to 60 percent in persons aged 50 and older. Over the same age span, the use of aspirin declined from 49 to 12 percent. The highest rate of self-poisonings was observed among married females under the age of 20. Unmarried girls in the same age bracket had a lower rate, which was statistically significant. Married women showed slightly higher rates than single women at all other ages, but both groups showed diminishing rates above the age of 25. Of the total number of persons in the study population, eight deaths were recorded (23).

In the Cardiff, Wales, area, all cases of intentional poisoning admitted for treatment between the years 1950 and 1965 were studied (26). During this period 1,730 deliberate poisoning episodes (classified as "serious attempt," "gesture," and "doubtful") were recorded, involving 1,541 patients. The number of female patients (1,048) was greater than the number of men patients (493) in all age groups, but the greatest number for both men and women was in the age group 25 to 45; these numbered 199 and 436, respectively. The next highest age group for women was 0 to 25 years and, for men, 45 to 65 years. Multiple admissions occurred in 14 percent of the serious attempts, 7 percent of the gestures, and 11 percent of the group classified as doubtful. Attempts were made to associate significant organic illnesses with the poisoning gesture. Twenty percent of the individuals did have some illness at the time of the episode. Twenty-six percent of the patients had been under psychiatric care, and 38 percent were known to have been suffering a mental illness of one kind or another, mainly depression before the episode. The agents used in these incidents were barbiturates, salicylates, and coal gas (carbon monoxide), which together accounted for 70 percent of the attempts. The number of fatalities that resulted from the ingestion of these and other agents was not specified.

The preference of barbiturates over other methods of committing suicide was pointed out in a report of an intercultural study of the suicide problem in Los Angeles, California, and Vienna, Austria. In one recent year, barbiturates and other drugs and chemicals were responsible for 30 deaths in Los Angeles and 31 in Vienna. The use of chemicals ranked after domestic gas and hanging in Vienna and shooting in Los Angeles.

The incidence of suicidal attempts (non-fatal) is difficult to establish. In a study by Parkin and Stengel (44) to determine the incidence of suicidal attempts in Sheffield, England, for the years 1960 and 1961, the following definition was adopted: "A suicidal attempt is an act of self-damage undertaken with the apparent intention of self-destruction, however half-hearted and ineffective." Results of their study showed that there were a total of 358 suicidal attempts admitted to hospitals in 1960 as compared to 39 actual suicides; in 1961, attempted suicides treated in hospitals totalled 159 as compared to 47 actual suicides. For 1960, 45 percent of the attempted suicides were attributed to narcotics, 15 percent to salicylates, and 20 percent to other chemical agents; for 1961, 40 percent were attributed to narcotics, 16 percent to salicylates, and 22 percent to other chemical agents. The remaining attempts for both years were due to hanging, slashing of the wrists, and other means.

The prevention of deliberate poisonings involves the application of numerous techniques such as proper counseling of persons with psychological problems and maintenance of proper controls over drug items. Persons ill from an overdose of a toxic chemical must receive proper diagnosis and treatment, and surveillance should be maintained over individuals in high-risk categories.

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MONITORING OF ENVIRONMENTAL TOXICANTS

John S. Wiseman

An understanding of the proper techniques for the collection, storage, and shipment of materials intended for analysis is necessary if environmental toxicants are to be accurately monitored. The quality of the analysis conducted on environmental samples is directly related to the collection procedure. Although the methodology discussed in this paper uses pesticides as examples, the overall principles apply to all environmental toxicants which are intended for analytical purposes. Monitoring of air, water, soil, adipose tissue, and body fluids will be discussed.

THE ANALYTICAL LABORATORY--A PANEL DISCUSSION

Frederick W. Oehme, William D. Rhoads, Herbert Starr, and John Tessari

A panel discussion covering four areas of laboratory management was conducted by representatives of independent laboratories. The discussion centered around the following topics:

Quality Control. Quality control is used to assure the output of reliable and valid analytical data. There are basically two general types of quality control: interlaboratory and intralaboratory. The former term indicates the involvement of two or more laboratories, providing a mathematical basis for the confidence placed in the results of sample analysis and an insight into analytical areas needing attention. An intralaboratory program provides a systematic system of methodology, use of standards, clean-up and related procedures within a laboratory to insure accurate analyses by each analyst. Each panel member discussed how his laboratory copes with each of these two general types of quality control.

Confirmation. The nature of analyses is such that interfering materials and artifacts are often observed and metabolic and decomposition products may also occur. Low concentrations of chemicals must be detected, measured, correctly identified, and confirmed by at least two independent analytical methods. Some methods, such as thin layer chromatography, paper chromatography, or p-values share the same physical property of partition in achieving separations of mixtures. They do not give independent evidence for the identity of a compound. Gas-liquid chromatography (GLC) retention times for a compound on different stationary phases are often highly correlated. Thus, the choice of confirmatory techniques should be carefully made.

General Problems. Laboratories involved in chemical analysis have their own unique types of problems. For example, a laboratory has the problem of separating polychlorinated biphenyls from chlorinated pesticides. Related problems may include sample collection techniques, storage of samples, transport and clean-up procedures.

Instrumentation. A variety of scientific instrumentation is being used today to obtain analytical data. Each panel member discussed the analytical instruments being used by his laboratory.

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POLYCHLORINATED BIPHENYLS IN SILAGE AND HUMAN MILK IN RURAL COLORADO

E. P. Savage, J. D. Tessari, J. W. Malberg, H. W. Wheeler, and J. R. Bagby

In 1966, Jensen first reported residues of polychlorinated biphenyls (PCB's) in tissues of fish and birds (1). Since then, PCB's have been identified in a number of different substrates, and the Food and Drug Administration has reported them in fresh fruits, vegetables, fish, poultry, eggs, milk, and in other food commodities (2-11). PCB's have also been reported in human adipose tissue and in human milk. Risebrough *et al.* reported PCB's in 16 human milk samples collected in California (12). Dyment *et al.*, in a study conducted in Texas, were unable to find PCB's in human milk samples from New Guinea and Texas (13). Skrentny *et al.* (8) had reported finding PCB's in silage stored in upright silos. The PCB's had leached into the silage from the sealants that had been used to coat the silo walls. These reports prompted us to question the presence of PCB's in human milk in areas of rural Colorado. Samples were collected from women living in and near Fort Collins and Greeley, Colorado. Since PCB's may be used in plastic and in upright silo sealants, we decided to check silage stored in both types of silo to determine if any PCB contamination had occurred.

Methods

A total of 39 human milk samples were collected from lactating mothers by manual expression and stored in 30 ml glass tubes fitted with plastic screw caps lined with teflon or aluminum foil. An epidemiological questionnaire was completed on each participant showing age, occupation, birthplace, race, years lived in Colorado, and if they had ever lived in communities larger than 50,000 population.

Silage samples were collected from 21 pit silos and 10 upright silos on ranches in Weld County, Colorado during the period of March to May, 1972. Ranches positive for PCBs were resampled during the summer of 1972 and these samples were also analysed for PCB's.

The silage samples were collected at random from the top, center, or bottom of the silage pits. In some instances, samples were taken from directly underneath the plastic. In the case of upright silos, the samples were taken from the dispensing nozzle of the flaker.

All silage samples were collected in 100 ml bottles with plastic screw tops lined with aluminum foil. The bottles were washed with hexane prior to collection.

Aroclor samples for standards were obtained from the Monsanto Chemical Co., St. Louis, Missouri. Standard iso-octane solutions of Aroclor 1254 and 1260 were prepared for gas chromatographic analysis so that 1 microliter of solution contained 500 picograms of Aroclor.

A MicroTek 220 gas Chromatograph equipped with a nickel-63 electron capture detector and Coulson Conductivity Detector was used for analyses. Columns used were: 1.5% OV-17/1.95% QF-1, 4% SE-30/6% (OV-210 and 3%

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OV-1). Solid support material was Chromosorb W, high performance DMCS 100-120 mesh. Temperatures for the Electron Capture Detectors were 300°C, injection ports and transfer lines 265°C, Columns 200°C, and Inlet 260°C. Thin layer chromatography equipment consisted of: 8" x 8" glass plates, 8½ x 4½ x 8½ developing tank, desaga/brinkmann standard mounting board, desaga/brinkmann standard model applicator, spotting pipettes, glass desicator, and ultra violet light.

The extraction procedure used was a modification of those described by Giuffrida _et al._ (14) and Curley and Kimbrough (15) and consisted of three parts: 1) the fat was isolated from the milk, 2) the PCB's were extracted from the fat, and 3) the extract was cleaned up. In our modification, samples of 15 grams of mothers' milk were placed into clean glass centrifuge bottles. Approximately 1 gram of glass wool was added to each sample. The purpose of the glass wool was to adhere to the coarse precipitate of the milk solids. After addition of 100 ml. of acetone, the milk samples were shaken for two minutes and centrifuged at 1000 rpm for 2 minutes. The acetone layer was then decanted into a one liter separatory funnel. After decanting, a volume of 25 ml of acetone was added to each sample and the extraction procedure was repeated three more times. All four extractions were then transferred into a liter separatory funnel. The portion of the milk sample that was coagulated was then extracted with 2-25 ml portions of n-hexane and centrifuged for 2 minutes at 1000 rpm. The two portions were then transferred into the one liter separatory funnel and 50 ml n-hexane, and 125 ml of a 2% sodium sulfate solution was added to the funnel. The separatory funnel was shaken manually for 2 minutes. The layers were then allowed to separate and the lower acetone-aqueous layer was drawn off and discarded. Another 125 ml of 2% sodium sulfate solution was added to the separatory funnel, shaken manually and allowed to separate. The lower aqueous layer was again discarded and the upper layer was poured into a 500 ml concentrator flask. The solvent flask was transferred to a rotary evaporator and the solvent extract was removed under water aspirator suction at 37°C until the solvent volume was reduced to approximately 2 ml. The sample extract was then taken through the Florisil procedure as described in the Manual of Analytical Methods (16).

The samples were analyzed by gas liquid chromatography. These analyses were followed by the TLC procedure for PCB's as prescribed in the Manual of Analytical Methods (16). The silage samples were analyzed by using the same methods.

Results

The epidemiological data for the eight participants with milk samples positive for PCB's are shown in Table 1. The levels of PCB's ranged from a low of less than 0.04 ppm to a high of 0.1 ppm. The range of age of the positive mothers was from 19 to 26 years. Three were born in Colorado, one each in Germany, Japan, Michigan, Ohio, and North Dakota. Seven were Caucasian and one Oriental. Five had lived in a city of 50,000 population or greater at some time during their lifetimes and three had not. Seven of the women were housewives, and one was a secretary. It is of interest that the two highest levels of PCB's were found in two housewives who had never lived outside of Colorado.

TABLE 1

Epidemiological Data for Lactating Mothers Positive for PCB Residues

Colorado - 1972

Age	Birth place	Occupation	Race	Years lived in Colorado	Lived in city larger than 50,000	Levels of PCB's
25	Colorado	Housewife	C	25	Yes	0.1 ppm
20	Colorado	Housewife	C	20	No	0.05 ppm
26	Michigan	Housewife	C	3	No	0.04 ppm
22	Germany	Housewife	C	3	Yes	0.04 ppm
19	N. Dakota	Housewife	C	7 months	No	0.04 ppm
25	Ohio	Secretary	C	2	Yes	0.04 ppm
20	Colorado	Housewife	C	19	Yes	0.04 ppm
23	Japan	Housewife	O	1	Yes	0.04 ppm

Of the 31 silage samples originally collected, two were positive for PCB's. The positive sites were resampled during the summer. Table 2 depicts the positive samples for PCB's collected from the two positive ranches. Site 20 is a ranch that feeds large numbers of feeder cattle. Silage is stored in pit silos and one upright silo at this ranch.

TABLE 2

Silage Samples Positive for PCB's

Weld County, Colorado - 1972

Site No.	Collection Date	Comments
20	5/31/72	Pit silage - 0.07 ppm
20	8/1/72	Pit silage - 0.04 ppm
20	9/8/72	Upright silo - trace
21	5/24/72	Silage & shelled corn from cattle trough - 0.06 ppm
21	8/3/72	Pit silage - 0.08 ppm
21	8/3/72	Shelled corn - 0.04 ppm
21	9/7/72	Shelled corn collected from truck - trace

The silage from Site 20 contained a residue level of 0.07 ppm on the first sampling and 0.04 ppm on the second sampling. Corn collected from an upright silo on the same ranch showed only a trace on PCB's and was at the lower levels of our PCB detection capability.

Site 21 is a dairy operation. The silage collected from the pit silo on this ranch contained PCB's at a residue level of 0.06 ppm. Collections of silage and shelled corn taken separately on the second collection had levels of 0.08 ppm and 0.04 ppm, respectively.

Discussion

Although PCB's were found in silage stored in both pit and upright silos on Colorado ranches, all of the positive samples were at very low levels, the highest level being 0.08 ppm. Only two (7.1 percent) of the ranches sampled were positive. One site had positive samples taken from an upright and a pit silo. Since this was a dairy ranch, 102 grams of milk were also collected and analyzed. This sample was negative for PCB's.

The length of time those positive for PCB's had lived in Colorado is of interest. Seven of the eight had lived in the state a year or longer. Two had never lived outside of the state and one had lived in another state for only a year. There were four other study participants who had never lived outside of Colorado who were negative for PCB's.

This study indicates that PCB's are found at very low levels in human milk in Colorado. The highest level was 0.1 ppm in a 25 year old housewife who has lived her entire life in the state. Six of the positive samples were at the low level of our detection capability at 0.04 ppm or less.

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INTERACTION OF PCB'S AND OTHER ORGANOCHLORINES WITH DUCK HEPATITIS VIRUS

Milton Friend

Introduction

The study of the impact of environmental pollutants on vertebrate populations has generally been oriented at the direct effects of single compounds. Thus, we study both the acute (LD_{50}) and the chronic (LC_{50}) toxicity of a compound, the compound's teratogenic, mutagenic, and carcinogenic potential, and, in the case of wildlife, its effects on reproduction. As diverse as these studies are, they provide only limited information on the hazards of a given compound.

Consider, for example, the variety of food consumed by the average person or wild animal within the relatively short span of a week. Each of these food items may contain its own particular combination of low levels of two or more chemical pollutants. The fact that these residues are at sublethal levels does not necessarily mean that they have no effect on the host, nor that the effect of combining them can be determined by simply adding up their relative hazards. I believe that biological interactions between chemical pollutants, and between these chemicals and various biological entities such as agents of disease represent the greatest hazard of many of the environmental pollutants studied to date. This situation is analogous to an iceberg, with the surface portion representing such overt, easily studied effects as mortality, and the submerged portion a variety of biological interactions induced by sublethal levels of chemical compounds (Figure 1). During the remainder of this paper, I will consider one of these interactions: that between chemical pollutants and agents of infectious disease.

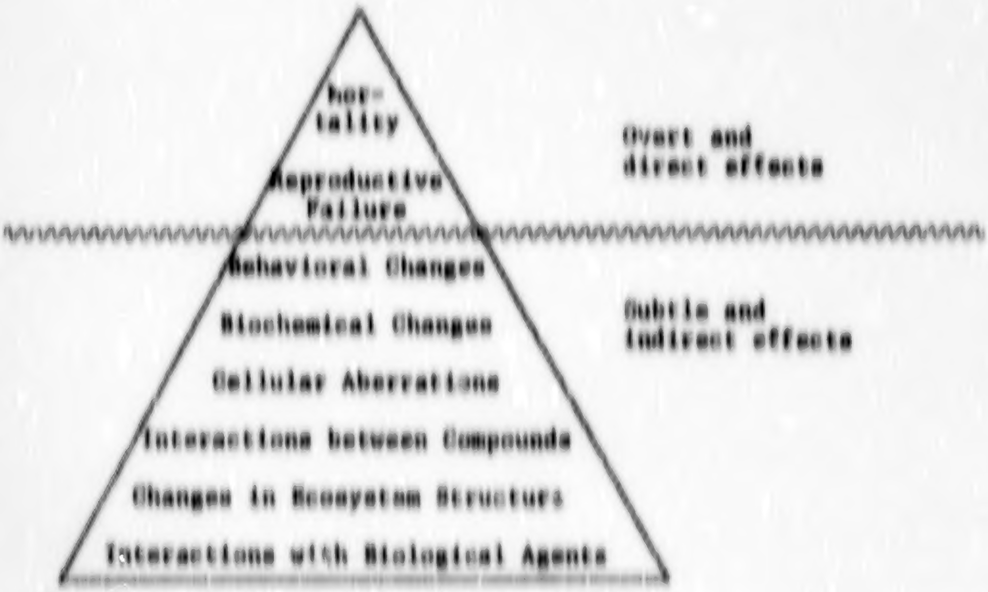
Mortality
Reproductive Failure
Overt and direct effects
Behavioral Changes
Biochemical Changes
Cellular Aberrations
Interactions between Compounds
Changes in Ecosystem Structure
Interactions with Biological Agents
Subtle and indirect effects

Figure 1. Environmental pollution iceberg.

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The problem of pesticide-disease interactions has usually been considered from the viewpoint of disease causing changes in the toxicity threshold of the pesticide for the host. However, the converse situation, pesticides altering a host's susceptibility to an infectious disease, has seldom been investigated. Since this is a session on polychlorinated biphenyls (PCB's) rather than pesticides, I will first describe an experiment combining Aroclor 1254 and duck hepatitis virus (DHV) and then use data for other chemical compounds to further illustrate this type of interaction.

Interaction Experiment

Protocol: Tucker and Crabtree (21) reported the minimum acute oral toxic dose of four different Aroclors to mallards, *Anas platyrhynchos*, as greater than 2000 mg/kg of body weight. Studies with other species have also indicated that Aroclors have relatively low toxicity for birds (13, 18, 19). On this basis, test levels of 25, 50, and 100 ppm of Aroclor 1254 were selected as sublethal levels for mallards and incorporated for 10 days into the diet of 10-day-old ducklings (25 per group); two similar groups of ducklings received untreated feed (Figure 2). On the 11th day of the experiment, five randomly selected birds from each treatment group were killed by decapitation and tissues collected for residue analysis and histology. The remaining birds were placed on untreated rations, transported about 3 miles to isolation units, and held in preparation for challenge with DHV.

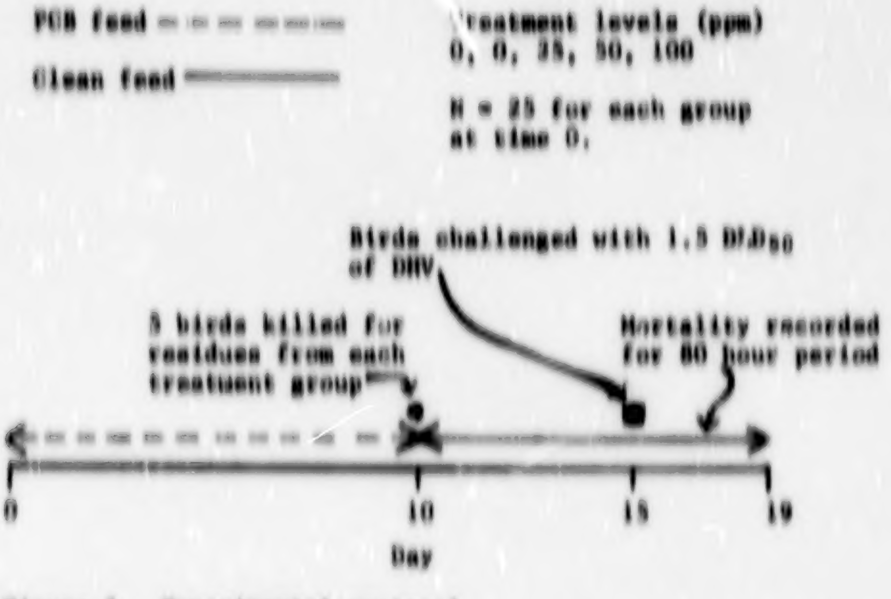
PCB feed
Clean feed
Treatment levels (ppm)
0, 0, 25, 50, 100
N = 25 for each group
at time 0.
Birds challenged with 1.5 DLD$_{50}$
of DHV
5 birds killed for
residues from each
treatment group
Mortality recorded
for 80 hour period
0
10
15
19
Day

Figure 2. Experimental protocol.

On the 15th day of the experiment, all but the "clean" control group were inoculated intraperitoneally with 1.5 DLD_{50} of DHV per bird. The inoculum was in the form of a 10 percent liver suspension; the clean controls received a similar dilution of a DHV-free liver suspension. Mortality was recorded hourly for the next 80 hours, except between hours 31 to 47 and 62 to 70. All birds that died within the 80-hour experimental period were necropsied and their brain tissues collected for residue analysis. After 80 hours, all surviving birds were killed and necropsied, and brain tissue was collected for residue analysis from five randomly selected birds in each treatment group.

Results. No birds died or showed clinical signs of intoxication during PCB feeding or during the interim period between PCB feeding and virus challenge. However, mortality following DHV challenge was significantly higher at the .01 level of probability (Chi-square = 7.49, 1 df) among ducklings that had received PCB in their rations (Table 1). No deaths occurred in the clean control group. The onset of mortality in all PCB-plus-DHV test groups began at least 8 to 16 hours earlier than in the DHV controls.

TABLE 1

Mortality among 10-day-old mallard ducklings, fed diets with or without PCB, following exposure to DHV.

Treatment*	Mortality**			
	Total[†]	Percent	1st death	Last death
Clean control	0/20	0	---	---
DHV control	3/21	14	62-70	73
25 PCB + DHV	7/20	35	31/47	75
50 PCB + DHV	13/20	65	55	76
100 PCB + DHV	8/18[††]	44	31/47	75

*PCB values in ppm; DHV = 1.5 DLD_{50}/bird.

**Observations were made hourly except between 31 to 47 and 62 to 70 hours.

[†] Number of deaths/total sample.

[††] Two birds were lost in a laboratory accident.

All birds that died had gross liver lesions indicative of DHV infection. No other gross lesions were observed, except for varying amounts of edema within the pericardium and thoracic cavity of some birds. This edema was not correlated with either PCB or DHV exposure. No significant histological changes were observed in sections of heart, liver, spleen, or brain tissues following PCB feeding. Histological changes in tissues of birds that died were indicative of DHV infections.

Residue analyses after 10 days of PCB feeding showed that mean brain residues in control birds were higher than in the 25 ppm test group (13 vs. 110 ppm) but lower than in the remaining test groups (Table 2). The source of this residue in control birds is unknown, but it was not the ration fed. Analysis disclosed less than 1 ppm (dry weight basis) of PCB in the control feed. Total residues in brain and liver increased with the level of PCB in the diet. The ratio of PCB in the brain to that in the liver was 1.03 for control ducklings and ranged from 0.37 to 0.89 for those receiving PCB rations.

TABLE 2

PCB residues in rations and tissues of mallard ducklings following 10 days of PCB feeding.

Treatment	Feed*	Tissue†		
		Brain	Liver	Liver plus Brain
Clean control	0.62	133	129	262
25 ppm PCB	27.88	110	241	351
50 ppm PCB	67.14	196	220	416
100 ppm PCB	105.90	205	557	762

* Residues on a dry-weight basis.

† Residues in ppm/gram of fat; all tissue assays represent pooled samples of 5 birds each.

PCB brain residues among birds that died following DHV challenge, with one exception, were higher than in birds killed at the end of the experiment (Table 3). The greatest brain residues of PCB were found in the birds that died the earliest after DHV challenge.

Discussion. The results of this study indicate that a PCB (Aroclor 1254) at relatively low levels in the diet (25 to 100 ppm) may increase the susceptibility of mallard ducklings to DHV. None of the levels of PCB fed resulted in detectable chemical intoxication, even with the physical stresses of weighing, handling, confinement, crowding during transportation, transportation itself, and relocation in a different environment. However, when the stress of an infectious agent was imposed on the birds five days later, there were two- to four-fold increases in mortality (14% among virus controls vs. 35% to 65% among PCB + DHV test groups), along with a reduced incubation period for the virus.

This type of response is somewhat alarming when one considers the variety of free-ranging wildlife exposed to PCB's. A survey of the literature through 1970 revealed 120 species in which PCB residues had been detected. Of these, 73 species were birds (Table 4). Similar increases in mortality was observed when DDT and dieldrin were used instead of Aroclor 1254 in interaction studies with DHV. In one experiment, mortality among virus controls was 7% and for ducklings pretreated with DDT or dieldrin, mortality increased to 47% and 57%, respectively (7).

TABLE 3

PCB residues in the brains of mallard ducklings following DHV challenge.

Treatment*	Time of Sample (Hours)			
	48**	48-70**	71-80**	80†
Clean control	---	---	---	60
DHV control	---	78(3)	---	59
25 PCB + DHV	293(2)	255(2)	286(3)	139
50 PCB + DHV	---	204(7)	128(6)	88
100 PCB + DHV	504(1)	226(3)	148(5)	163

* PCB values in ppm; DHV = 1.5 DLD$_{50}$/bird.

** Pooled samples from birds that died; figure in parentheses indicates sample size.

† Pooled samples from birds killed at end of experiment; N = 5 for each sample.

The mechanism responsible for this synergism between DHV and the 3 organochlorines studied is unknown. An apparent synergistic response resulting in deaths of rats after exposure to both chlorinated diphenyl and carbon tetrachloride was reported 35 years ago (6). Since carbon tetrachloride, like PCB and DHV, is capable of producing acute toxic changes in the liver (20), these findings suggest that the liver is involved in the synergistic responses reported. Studies with rats and other laboratory mammals have disclosed persistent liver lesions following PCB exposure. These lesions consist primarily of fat droplets generally scattered throughout the lobule, atrophy, necrosis of liver cells, and central fatty degeneration (11, 17). The lack of definite histopathology due to PCB in the present study may be a result of species differences, low dosage levels, a relatively short feeding period, or some combination of these factors. Some birds showed swelling of hepatic cells, loss of cell nuclei, some inflammatory response, and what appeared to be fat droplets, but treatment groups could not be distinguished from each other or control groups on this basis.

Certain diseases, such as obstructive jaundice, untreated alloxan-induced diabetes, hepatic tumors, and partial hepatectomy, because of their known effect on the activity of liver microsomal enzymes, might be expected to interact with pesticides (5). Conversely, since most toxicants are detoxified in the liver, they might influence the course of a disease involving agents or conditions that affect the liver tissue. DHV is such an agent, and in the presence of certain pollutants, apparently interacts with them to the detriment of the host.

Other evidence for increased host susceptibility to disease following pesticide exposure can be found in the literature. For example, Wassermann _et al._ (22) found significant reductions in antibody production among rats fed DDT, and Gabliks and Friedman (9) found increased susceptibility of insecticide-treated cell cultures to polio virus, presumably as a result of inhibition of interferon activity.

TABLE 4

A tabulation of wildlife species with PCB residues (through 1970).

Classification	No. of Species
Birds	73
Fish	
Saltwater	32
Freshwater	5
Shellfish	4
Mammals	
Marine	5
Terrestrial	1
Total	120

Literature reports of possible pesticide involvement in wildlife disease outbreaks are more numerous for fish than for warm-blooded species. Allison _et al._ (1) concluded that DDT increased mortality in cutthroat trout (_Salmo clarkii_) during toxicity trials by reducing resistance to disease. Schoenthal (cited by Cope (5)) observed a higher prevalence of furunculosis and fungus disease in DDT-treated salmonids than in control fish and Lowe (16) found brain parasitism in Sevin exposed spot (_Leiostomus xanthurus_), but not in control fish. According to studies at the Fisheries Research Institute at Vodnany, Czechoslovakia, organic pollution promotes infectious dropsy, ulcers, and furunculosis in fish, while pollution of inorganic origin can result in blindness, increased intestinal parasitism, and sexual sterility (2).

Gilbert (10) concluded that mink (_Mustela vison_) that died of Chastek's paralysis in his study, were stressed by DDE to the point where they were more vulnerable to other agents. On the basis of epizootiological data, Friend and Trainer (8) suggested that epizootics of Type E botulism in water birds on Lake Michigan and avian cholera in waterfowl, especially in California and Texas, are field situations where pesticides may be important factors in the epizootiology of wildlife disease.

Conclusion

Finney (6) has stated that "any attempt to understand fully the toxic action of a group of insecticides or fungicides must ultimately involve a study of their behavior when two or more are applied in mixture." Data presented here emphasizes that interactions of chemicals with other biological agents must also be studied before we are able to understand fully the potential hazards of these chemicals. One need not be a biologist to appreciate that exposure to environmental pollutants in nature is continuous and insult from multiple agents (chemical, physical, and biological) during the life span of an individual is the rule rather than the exception. Lodge (15) recognized this problem in stating that while the study of synergism applied to environmental pollution has scarcely begun, the problems that must be dealt with are alarmingly advanced.

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CARBON MONOXIDE AS A NATIONAL PROBLEM

Richard E. Gallagher

It has been conservatively estimated by public health officials that more than 10,000 persons per year are overcome or seriously incapacitated due to exposure to hazardous levels of carbon monoxide gas. More significantly, mortality records reveal that an average of 1,400 persons per year die as a result of carbon monoxide (CO) poisoning.

Analysis of mortality statistics reveals that approximately 70 percent of all fatal injuries of this type occur in the home environment. It is also of value to note that more than 50 percent of these injuries occur during what is commonly known as the heating season.

The reported numbers of deaths and injuries due to CO are believed to be only the "tip of the iceberg." Public health and medical authorities believe that many more injuries and deaths occur as a result of carbon monoxide poisoning but are not reported because CO is not suspected and may not be recognized. Carbon monoxide victims are often diagnosed and treated for such varied ailments as food poisoning, influenza, sinusitis, pneumonia, and typhoid fever, as well as cardiac failure, viral infection, meningoencephalitis, acute alcoholism, and mental illness. According to health authorities in one state, a person was admitted to a mental hospital four times before he was diagnosed as having CO poisoning.

For more than a half century, the occupationally related hazards of carbon monoxide have been well known. However, it has only been recently that injury and death resulting from exposure to hazardous levels of carbon monoxide have been of concern and accepted as a significant public health problem.

Our civilization could not exist as we know it without the combustion of fuels. Carbon monoxide, one by-product of combustion, is increased when there is incomplete combustion. It comes from our non-nuclear power plants, from our automobiles, heating appliances, fuel-fired clothes dryers and kitchen ranges. In reality, varying concentrations of carbon monoxide continuously surround us.

CO weighs slightly less than air (specific gravity 0.9671), yet it has been calculated that not less than 102 million tons of this toxic gas annually dumped into our nation's living, working, and recreational environment (7). In commercial and industrial work areas, CO often reaches levels detrimental to health. One study reported in the Archives of Industrial Health found the CO level in a large cold storage plant had reached 400 ppm (5). Another, conducted by Dr. Stephen Ayers, reported CO in excess of 100 ppm in New York traffic terminals, with occasional concentrations of 200 ppm (2). A 30-daystudy conducted in the GSA garage of a federal building found CO readings as high as 400 ppm for short periods of time and an average of over 50 ppm for a 72-hour period (8).

CO is an odorless, tasteless, and colorless killer which combines with the hemoglobin in our blood, decreasing the capability of the blood to carry life-giving oxygen. It does this by combining with the red blood cells. In fact, the affinity of carbon monoxide for hemoglobin is over 200 times that of oxygen.

To bring our exposure to CO into perspective, reports state that concentrations of COHb as low as 5% to 10% have been associated with adverse health conditions. These conditions are delayed reaction time, impaired vision, and artherosclerotic changes in the main trunk of the aorta (3, 4,6).

Professor Astrup and his Danish research team reported in the March 1972 issue of Scanorama that "workers in steel mills, traffic tunnels, garages and some other environments get more than their share of noxious fumes. It might be that as little as 2% COHb continuously or intermittently for several hours a day could be harmful. If a large-scale experiment with 200 rabbits proved this, then I think that 2% COHb in man also would have a harmful effect." (1)

In this study, COHb levels of around 18% (approximately 200 ppm during gestation) considerably increased the neonatal death rate of rabbits, especially on the first day of life. The rabbits bore 120 young; only one rabbit from the unexposed control group was stillborn, whereas 16 from the same size study group were stillborn or died the first day. Five of the study group were born malformed (with shorter limbs, for example), a phenomenon that was never seen in previous experiments in the Danish lab. After 6 days, the death rate was twice as high in the study group as in the control group. The experiment was repeated with only 8% COHb blood levels (a level constantly carried by many heavy smokers), and a similar tendency was noted in the results (1).

Many researchers believe that CO crosses the placenta and replaces enough vital oxygen in the blood of the fetus to interfere with normal central nervous system development. When CO affects the central nervous system, a person is more likely to have an accident while driving, during recreation, at work, or at home.

The 1971 Report of the Surgeon General on Smoking indicated that women who smoke or receive low levels of CO continuously or intermittently for several hours a day from any environmental source tend to have more complicated pregnancies and produce more premature lightweight babies. These findings were recently supported by a mother and child survey of 350 women at Yale-New Haven Hospital in Connecticut.

Since injuries and deaths resulting from exposure to unsafe levels of carbon monoxide in the home are often of the same magnitude as many of the communicable diseases, it is fitting that public health officials should concern themselves with this problem, channeling a considerable amount of their energies and expertise toward dealing with CO poisoning in a manner similar to that being used in combating communicable diseases.

It is imperative that public health workers recognize at the outset that epidemiological methods and techniques which will be used in dealing with the problem of carbon monoxide poisoning at the domestic level must involve the study of influences of many kinds, including the characteristics of a host population, specific agents, and biological, social and physical environment. Keeping this in mind, I would like to share with you the experience of one city-county health department in their effort to combat CO on a community-wide basis.

In 1964-65, the Memphis and Shelby County Health Department conducted a study of carbon monoxide in homes. The study was based on investigations of incidents of carbon monoxide exposures obtained from the following sources:

1. Reports from the emergency rooms of Memphis city hospitals.
2. Complaints of fumes reported to the Memphis Light, Gas, and Water Company.
3. Random home surveys in selected neighborhoods.

This study revealed that the following factors contribute to unsafe levels of carbon monoxide gas in the home:

1. Flame impingement on metal surfaces.
2. Clogged air mixing inlets.
3. Poorly designed and/or improperly modified appliances.
4. Failure to vent gas appliances.
5. Leaks in combustion chambers and heat exchangers.
6. Improperly installed and/or clogged vent pipes.
7. Malfunctioning fuel regulator valves.
8. Oxygen depletion and negative pressure situations.
9. Improper use of fuel (charcoal, coke, fuel oil, L.P. Gas, etc.)

The following recommendations were put forth as a result of this study:

1. The installation of all combustion appliances should be by qualified persons (often these are not available in small towns resulting in plumbers and "do-it-yourselfers" doing the installation).
2. Heating systems should be checked and given routine preventive maintenance at least annually by qualified persons ("qualified" is difficult to define).
3. All combustion heating appliances should be vented to the outside of the building.
4. Only the fuel designated by the manufacturer should be used.
5. Only appliances that bear the seal of a recognized approval agency should be used.
6. Open flame space heaters that are not adequately guarded, vented and equipped with fuel pressure regulators should not be used in homes.

7. Provide source of make-up air to avoid the problem of negative pressure.

Analysis and interpretation of this preliminary study immediately showed that the staff from the Memphis and Shelby County Health Department was not large enough to deal with a public health problem of the scope and magnitude that was identified.

A manpower survey was made by representatives of the Division of Injury Control to determine if other assistance was available in Memphis and Shelby County to deal with the problem of carbon monoxide poisoning in the home. It was determined that there were seventeen other official and non-official agencies in the city and county which had interest in the total accidental injury problem similar to that of the Division of Injury Control.

Each of these agencies was provided with packets of information on carbon monoxide poisoning. Each was requested to contribute in a manner consistent with its ongoing functions. Also, it was determined that public service time was available at no cost through all local radio and television stations. Even more importantly, the aid of all sanitarians, public health nurses, and investigators was solicited in developing a manpower source capable of dealing with the carbon monoxide problem in Memphis and Shelby County.

One other important element which was extrapolated from this preliminary study was that there was little, if any, community knowledge about the total problem of carbon monoxide poisoning in the home. To assure maximum receptivity and response on the part of other agencies, co-workers, and the community at large, community information sessions were held for all interested groups and organizations. An in-depth training session was provided for all staff members of the health department. This session sought to maximize the effectiveness of health department representatives in identifying those situations in the home which are indicative of carbon monoxide poisoning by orientating them in the basic principles of carbon monoxide poisoning.

All representatives from other agencies which were involved in some phase of accident prevention were invited to a joint meeting. Each listed how his agency could contribute to the total community effort.

To assure maximum dissemination of community information, selected radio and television spot announcements using all local voices were recorded and distributed to all stations.

Since the initiation of this program, the county's death and injury rate due to CO poisoning has declined despite an increase in population. The radio, television, and other news media continue to provide public service time to support a continuous county-wide CO educational program. CO poisoning prevention programs are being conducted by many of the seventeen original cooperating agencies and coordinated by the local health departments.

CO in any community is one element of a total environment, and as such, may combine with other stressors with a synergistic effect. Exposure to non-lethal levels of CO in a dwelling or outdoors in the ambient air is not a desirable condition; however, the situation becomes worse if the exposed person is at the same time subjected to the additional stresses that are common in many of our inner city neighborhoods today. These stresses include crowding, lack of privacy, lead poisoning of children who eat peeling flakes of lead-based paint, congestion within the neighborhood, lack of accessibility to essential health and medical services and to recreation areas. In addition to these stresses, a deprived living environment often includes inadequate lighting, poor ventilation, excess noise, wide variations in temperature, nauseating odor, poor sanitation, rats, burn hazards, and a host of other potential environmental and health hazards.

We have known about the health hazard associated with CO for many years. Until 5 or 6 years ago, industrial standards prohibited an individual from working an 8-hour day in an environment containing more than 100 ppm of carbon monoxide. Increased scientific knowledge has created a concern for exposure to excess amounts of CO and as a result the industrial standard has been reduced from 100 ppm to 50 ppm for an 8 hour day.

There is some indication that an exposure of 8 or more hours to a CO concentration of 10 to 15 ppm has been "associated with adverse health effects as manifested by impaired time interval discrimination." (4) In other words, with exposure to low levels of CO, your likelihood of having an accident while driving, at work, during recreation, or even at home, may be increased.

There are those who question the current industrial standard of 50 ppm. the H.E.W. publication "Carbon Monoxide--a Bibliography with Abstracts" (9) indicates that even 10 to 15 ppm over 8-hour periods does the human organism no good. Duration of exposure to CO gas is definitely a factor. For this reason we are concerned with the mother and child, the ill and aged who spend 24 hours each day in a low concentration of CO.

It would appear that a maximum acceptable concentration of CO in dwellings which would result in no adverse effects on the health and well being of the occupants should be established and enforced. The Bureau of Community Environmental Management is in the process of selecting an ad hoc committee of the most prominent scientists and researchers from the U.S. and Canada to assist us in establishing a maximum permissible CO level for the home environment. It is hoped that this standard will be established by the beginning of the 1972-73 heating season. However, a standard is of little value unless it is applied at the local level.

Now, let us consider some of the essential components of a comprehensive action program at the State and community level. First of all, such a program should include a surveillance function. This will keep you informed of where the problems are, who is affected, and new problems that may be emerging, and will also provide a baseline against which progress can be measured. Mechanisms can be established that will ensure prompt notification to the health department of all incidents involving high

high carboxyhemoglobin levels in people and high CO levels in dwellings. This can be done through the cooperation of hospitals, fire and police departments, gas utility companies, coroners' offices, and others. Surveys of homes using CO detection equipment or screening devices should become a routine part of housing inspections.

The second part of a community action program should be the prompt investigation and reporting of cases of CO poisoning, fatal and nonfatal. We should learn all we can about the individuals involved, the emitting sources, how the problem relates to existing codes or regulations, and the general environment in which the events take place. This information is essential in developing countermeasures and in determining the direction of an action program. In the case of malfunctioning heating and cooking appliances, it is important to know the name of the manufacturing company, model number, serial number, date of installation, what maintenance had been provided, and how the installation was accomplished. Health and medical histories and other background information about the individuals involved are important. Identification of all CO emitting sources, air exchange characteristics, and other stresses in the environment includes other essential information to be obtained. This requires a referral system and the cooperation of several community agencies.

Implementing control measures involves a wide variety of activities. Codes and ordinances must be adopted, checked, revised if necessary, and enforced. For example, what does your community require about the sale, use, and installation of space heaters? Does it require specific periodic inspection of furnaces and chimneys? Are air samples in occupied dwellings a part of housing inspections? The answers to these questions must be in the affirmative to effect sound control measures by an action program.

Education directed at various segments of the community is a must. The health department staff and housing inspectors are key participants in the program and must be informed and motivated. The index of suspicion of medical and paramedical professions of CO poisoning must be elevated and periodically stimulated. The utility companies must participate. Plumbers and other contractors need to be informed. Various segments of the general public must be informed and motivated.

Behavioral scientists tell us that before a person decides to act positively to protect his health, three conditions or beliefs must be satisfied. These beliefs related to CO are 1) that he can be susceptible to CO poisoning in his home environment, 2) that exposure to the gas could have serious consequences for him or his family, and 3) that recommended control measures will be effective. The absence of any one of these beliefs almost assures that no decision to act will be made.

Educational efforts directed at and involving children can be very effective. The concept that accidents are caused and are not "acts of God" can easily become an important part of a youngster's intellectual development.

Two community projects that might be considered as part of a broader community program that contain elements of surveillance, epidemiology, and implementation of control measures are the following:

1. Local industries, as part of their off-the-job safety program, could be motivated to use CO detector screening tabs in the manner described by the Argonne National Laboratory during its 1969 project. In this project, workers were informed about potential CO hazards in homes and automobiles. They were given the opportunity to place small CO detector tabs (screening devices) in their automobiles and homes for several days. A short questionnaire was distributed to be returned by each user with the tabs. Contacts were made with the persons reporting or returning darkened tabs (positive CO finding) and, where possible, the presence and concentrations of CO were evaluated by sampling with portable CO detector instruments. In the case of automobiles, testing was conducted in an area adjacent to the laboratory. Twenty-seven percent of the individuals participating obtained positive indications of CO in either their homes or their automobiles. This project resulted in corrective actions being taken, such as repairing automobile exhaust systems, replacing home furnaces, and improving exhaust ventilation on home gas heaters. It appears that since the CO tabs are generally reliable as a screening device and relatively inexpensive (about 50¢), this project could be a valuable part of any occupational safety off-the-job program during the winter months.

2. BCEM, in cooperation with local units of government, is planning to conduct a CO poison prevention demonstration project in several communities this year. Many of the study areas will be in model cities and in high risk neighborhoods similar to those in your area. The project will be conducted something like this: After permission is received from parents, children in select age groups will, upon arrival at school, be checked for elevated carboxyhemoglobin levels. The parents of those children having elevated levels will be contacted. The sources of CO in their home environments will be identified and appropriate action taken to correct or remove these hazards. In other areas, a number of homes will be screened for CO. In those homes found to contain a hazardous CO level, the emission sources will be identified and action will be taken to correct the situation.

It seems that either of these two projects has a lot to offer in increasing community awareness, educating residents, and minimizing the CO problem by locating and correcting hazards.

Evaluation of the impact of the community action program can be obtained in terms of reduction in CO-induced morbidity and mortality, in the numbers of hazardous situations identified and eliminated, and in increased community awareness and concern about the problem. This will be evidenced by individual and group actions, such as upgrading existing regulations or establishing new ones.

CO community action programs offer something which seems to be lacking in many other community health programs. They offer approaches to the problem which are practical and simple to do. They are easily understood and consequently appreciated by residents. This is especially true since the hazard has something of an aura of mystery about it--CO cannot be seen, smelled, tasted, or felt; and it strikes without warning. Community prevention programs will contribute to improved public relations for your department and will stimulate the morale of staff members who will readily see the tangible benefits in preventing ill health from an obvious environmental stressor.

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CARBON MONOXIDE POISONINGS AT HIGH ALTITUDES

Freeman D. Fowler

A knowledge of the causes, effects, and treatment of carbon monoxide poisonings in man has been gained during thirty-six years as a medical practitioner in the small mountain town of Idaho Springs, Colorado.

Idaho Springs is located in a canyon at an altitude of 7540 feet near two major highways that cross the Continental Divide. Highway grades in and near Idaho Springs are very precipitous and one hill near the community increases 1500 feet in altitude in a distance of three miles.

Tourists who come to Colorado's high country to escape the heat of their home communities seem to be the most frequently affected by carbon monoxide poisoning. They come in short pants and get up on Berthoud or Loveland Pass where it is cold, so they close the car windows and may begin to experience the symptoms of carbon monoxide poisoning.

The risk of carbon monoxide poisoning is greater above 7,000 feet altitude than it is at sea level because the lack of sufficient oxygen at high altitudes results in poor combustion, causing motor vehicles to emit much more carbon monoxide. At the same time, the human body is deprived of about half of the blood's normal oxygen carrying capacity, thus making the body unable to cope successfully with only half as much carbon monoxide.

Important aspects to be considered in carbon monoxide poisonings are: the source of carbon monoxide, the effect of weather and barometric pressure, and other time and place considerations.

CHEMICALS IN THE WATERS OF THE ROCKY MOUNTAIN REGION

Charles G. Wilber

For those who are not familiar with some of the unique factors associated with natural waters in the Rocky Mountain region, it is important to realize that there are few rivers, streams, or ponds in the Rocky Mountains that have not been modified by human endeavors. Streams are rechanneled, diverted, diluted and otherwise modified--primarily for purposes of irrigation but also for purposes of domestic water supply. These modifications sometimes are minor; at other times they are of a major nature with significant consequences. In many instances, the detailed changes, with respect to time, etc., have not been recorded in such a way as to permit the scholar to get a clear historical picture of some of these modifications.

One of the changes which is common is the transmountain diversion of water. For example, at the end of 1957, an average of about almost 500,000 acre feet and over 37,000 tons of dissolved solids were diverted annually from the upper Colorado River Basin over to the eastern slope of the Rocky Mountains. Of these total amounts, over 350,000 acre feet of water and nearly 18,000 tons of dissolved solids were diverted from the Colorado River and its tributaries above the Gunnison River. This area of the river is sometimes referred to as the "Grand River" by so-called purists.

Such large diversions across the mountains can result in significant changes in the adjusted average for dissolved solids concentration when the flow of a stream or a complex of streams is depleted by diverting significant amounts of water across the Continental Divide:

> For example, the transmoutain (sic) diversions from the Colorado River above Hot Sulphur Springs, Colorado, have decreased the average annual water discharge from about 417,300 acre feet in 1914 to about 176,000 acre feet in 1957; have decreased dissolved-solids discharge from about 34,900 tons to about 18,260 tons; and have increased the weighted-average concentration from about 61 to 76 ppm.

Calculations have indicated that the diversion of water eastward across the mountains has increased the adjusted average concentrations of dissolved solids at Lee Ferry, Arizona, by about 3.4 ppm for every 100,000 acre feet of water diverted across the mountains.

It is known that nearly 300 inorganic chemical species may exist in the fresh water environment. However, a detailed survey of the published literature indicates that only 87 species of inorganic chemicals have been identified. A wide distribution of these chemicals in concentrations in drinking water and in polluted water is reported. There are data available on acute toxicity, chronic toxicity, carcinogenicity, mutagenicity, and teratogenicity. The design of the vast majority of reported toxicological experiments is such as to frustrate attempts to extrapolate this information to problems of human health. In most cases, the data were collected with reference to the adverse effect of these

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chemical species on aquatic organisms. Man was not included as a possible target organism in most of the considerations.

In the literature, there are numerous correlations made of minimal lethal dose versus maximum concentrations reported in fresh water, and of minimum chronic toxic dose versus maximum concentrations reported in drinking water, etc. However, the method of reporting and the mathematical manipulations used do not aid, in a direct and meaningful way, the expert who is charged with problems of human health.

It is long overdue that concern for fish, worms, snails, and fly larvae be moderated and more direct attention be paid to the impact of chemical species in fresh water, as individual chemicals and as chemical complexes, on the human organism both acutely and chronically. Admittedly, such an orientation is extremely difficult to implement but it is essential if we are to make any progress with respect to environmental pollution problems.

It is important to realize that an assortment of chemicals in unusual concentrations in water can modify drastically the biology of a species without directly killing that species. For example, fish are known to respond physiologically to a combination of sewage pollution and low levels of dissolved oxygen. The effects that are seen include decreased swimming stamina and respiratory efficiency. In addition, there is a decrease in oxygen consumption and an elevated blood and muscle lactate. Under these conditions, there is a suppression of urine flow and an increase in the amount of ammonia excreted. The elevated ammonia excretion is especially pronounced in the presence of environmental ammonia. Careful study indicates that long-term disruption of the normal hematological picture in the fish and of the lipid metabolism are identified. Most of the effects are observed at dissolved oxygen concentrations just below 5 mg/l, with the exception of the synergistic effects between ammonia and low dissolved oxygen which are observed at somewhat higher concentrations of dissolved oxygen (6).

When one considers a complex chemical mixture such as fresh natural water, the phenomena of synergism and antagonism must be recognized.

A synergist is a chemical agent which by itself may be mildly effective biologically (e.g., non-toxic) but when combined with some other chemical species results in a greatly increased biological action (e.g., greater toxicity, more pronounced rate change of a function). The sum of the individual actions of the two chemicals is always less than the action of the mixture of the synergists. The phenomenon resulting from the action of synergists is called synergism, activation, or potentiation (18).

> The phenomenon of synergism is one of great importance in any biological consideration of water pollution. It is a factor in evaluating the biological effects of metals polluting natural waters. Few polluting effluents are solutions of a single pure chemical; most are complex mixtures which present every opportunity for interactions such as antagonism and synergism.
>
> Copper and zinc salts are known to have a synergistic action when tested against some fresh water fishes. Studies using

the rainbow trout show that the manner in which toxic action is exerted is similar for zinc and copper.

In low concentrations of a combined mixture of zinc and copper sulfates, the toxic action of the mixture can be predicted from the sum of the toxic actions of the individual salts, that is, an example of "similar joint action."

At high concentrations in soft water, the toxicity of a mixture of copper and zinc sulfates for rainbow trout is greater than can be predicted from the sum of individual toxicities, that is, synergism occurs. The levels of concentration of copper and zinc at which synergism occurs are so high as to suggest that under reasonable conditions to be expected in polluted waters, similar joint action will occur; synergism will be of no practical importance.

Young Atlantic salmon, *Salmo salar*, are sensitive to copper and zinc. The incipient lethal level (concentration below which the experimental fish survive indefinitely) for copper is 48 µg/l; for zinc, 600 µg/l. Above the incipient lethal level, a plot of log concentration of metal against time to 50% mortality gives a straight line for both zinc and copper.

If both zinc and copper are present in a mixture of salts, young Atlantic salmon die much faster (by a factor of 2) than one would anticipate from a simple summation of individual toxicities. Young temperature-acclimated salmon survive a given concentration of zinc only one-fourth as long at 15°C as at 5°C. The incipient lethal level for zinc is elevated about 1.5 times at 5°C.

Is only dissolved zinc toxic, or is zinc in suspension also toxic to fishes? Studies using rainbow trout as the test organism suggest that suspended zinc is toxic. Other investigations using the Atlantic salmon indicate that only dissolved zinc is toxic. Additional research on this problem is needed. Answers are required for all metals which are potential water pollutants to clarify whether suspended, dissolved, or both forms of an element are toxic. Moreover, data are required to clarify the role of precipitated metals in any kind of toxic interaction. Studies at the molecular level of the mechanism of toxic action of these metals on aquatic animals should be encouraged.

Antagonism is essentially the opposite of synergism. The total effect of the individual chemical agents is less than the sum of the separate effects when taken independently. The mechanism is obscure. Perhaps it is simply the precipitation of the active species in an insoluble form.

Industrial effluents contain organic chemicals, inorganic chemicals, or both. Synergism and antagonism probably are involved in the final overall biological action of the effluent. The quantitation of these phenomena under field conditions (except under special circumstances) is difficult. Thus, little is known about synergism or antagonism as it occurs in receiving waters into which industrial effluents flow.

Stream Flow

In order to understand some of the problems associated with studies on the chemistry of Rocky Mountain waters, one must recognize that the natural yearly flows vary widely from virtually zero to impressive floods. Moreover, man has manipulated these running waters in a bewildering fashion. Flowing waters are dammed, diverted, run into foreign channels, and otherwise modified to meet the needs of man--primarily agricultural. The Cache la Poudre River near here is a controlled flow stream whose waters are bastardized by periodic injections of foreign water as required for irrigation. These manipulations complicate ones understanding of the chemical dynamics of Rocky Mountain waters.

The following table illustrates the flow pattern of the Colorado River:

	Mean Discharge		Extremes, CFS	
Location	CFS	Acre ft/yr	Max.	Min.
Grand Lake, Colorado	98	70,880	1,840	1.6
Glenwood Springs, Colo.	2,812	2,036,000	30,100	286
Cameo, Colorado	4,032	2,919,000	36,000	700
Utah State Line	6,088	4,408,000	56,800	960

Another way of illustrating variability of stream flow is through the use of the average discharge and the standard deviation. The following table illustrates these facts:

Location	Ave. Discharge CFS	St. Dev. CFS	Coef. of Var.
Fraser River	41	12	.29
Colorado River	676	189	.28
Blue River	116	31	.27
Rifle Creek	25	4	.16
Parachute Creek	30	20	.67
Gunnison River	2,600	1,040	.40
San Miguel River	374	158	.42
Yampa River	472	126	.27
Elk River	356	9[illegible]	.26
White River	638	142	.22
Little Snake River	547	185	.34

Uncontrollable factors which face the biologist who is attempting to evaluate the role of chemicals in Rocky Mountain waters include the fact that so many of the major streams and rivers in the Rocky Mountain region have an extremely variable flow. The Colorado River is an example which may be useful to set the stage. At Glenwood Springs, the average flow of the Colorado River, for over a 60-year observation period, is 2,812 cubic feet per second. However, the range is rather remarkable--from a maximum discharge of over 30,000 cubic feet per second to a minimum of around 266 cubic feet per second. Farther downstream, at Cameo, Colorado, the average discharge observed between 1933 and 1957 is a little over 4,000 cubic feet per second. The range again is remarkably wide; the maximum discharge is 36,600 cubic feet per second, the minimum 700 cubic feet per second. At the Colorado-Utah State Line, the average flow of the Colorado River measured for a short period of time in the 1950's was over 6,000 cubic feet per second. The extremes went from a maximum discharge of nearly 37,000 cubic feet per second to a minimum of about 1,000 cubic feet per second.

Measurements have been made of the Yampa River flow at Steamboat Springs; over a half century, the average discharge is somewhere around 500 cubic feet per second. However, the range is from a high value of nearly 7,000 cubic feet per second to a low value of 4 cubic feet per second. Further downstream at Maybell, the Yampa River (which at that point drains an area of about 3400 square miles) has a 50-year average discharge of 1,587 cubic feet per second. The maximum discharge recorded is nearly 18,000 cubic feet per second, the minimum 2 cubic feet per second.

With the enormous ranges in stream flow which are characteristic of these Rocky Mountain rivers, it is obvious that the chemical picture changes drastically over a normal course of time. In fact, a number of considerations lead to the suggestion that the normal, natural changes in stream flow are of such a magnitude as to overshadow and virtually engulf any man-made changes which might result from industrial operations or, indeed, from reasonable agricultural operations.

In all probability, future manipulations of these rivers by man will be in the direction of stabilizing, over the course of time, the stream flow. When the discharge rate varies so extremely, it is evident that the aquatic organisms are subjected to overwhelming stresses. The fact that these stresses have occurred for millions of years suggests that the native organisms have adapted in some way to these regular insults. However, it also seems important to recognize that for the best possible multiple, compatible use of these rivers for domestic purposes, for industrial purposes, for agricultural purposes, and for recreational purposes, a more stabilized discharge rate seems desirable.

Trace Elements

Heavy metals in United States waters are a matter of widespread concern. Some of this concern is hardly warranted despite the sound and fury accompanying it.

Most States in the Union have established acceptable levels for heavy metals in water where such standards can be arrived at in a rational manner.

According to the Environmental Protection Agency (5), Colorado has adopted the following standards:

Metal	Level in mg/liter	Applied to
Cadmium	0.01	Water supply
Chromium	0.05	Water supply
Lead	0.05	Water supply
Silver	0.05	Water supply
Zinc	0.05	Water supply

Other Rocky Mountain States have established standards as follows:

Metal	Level in mg/liter	Applied to
	Arizona	
	No specific criteria	
	New Mexico	
All toxic materials	Not to exceed 10% of 48-hr TLM	All classes of water
	Utah	
	USPHS Standards	All uses
	Wyoming	
	No specific criteria	

The Environmental Protection Agency (5), through its Division of Water Quality Standards, claims that:

> "Mercury, silver, arsenic, cadmium, chromium, copper, lead nickel, and zinc are heavy metal compounds present in our waters and toxic to man in varying degrees. They are serious pollutants because these stable compounds have persistent and toxic effects for many years following deposit. The heavy metal compounds chromium, cadmium, mercury, and lead have no known biological function in animal life and can act synergistically with other substances to increase toxicity. Marine organisms, especially shellfish, readily take up and concentrate these heavy metals, which are thereafter ingested by man. Once in the human, their toxic effects are cumulative and are harmful to the degree that the dosage and resultant concentrations approach a lethal threshold. The fishery industry has sustained economic losses in recent years when unacceptable levels of mercury or other heavy metals were discovered in fish from contaminated waters, provoking government condemnation of the affected catches. Fishing waters have been closed to fishermen, cutting them off from their livelihood."

Lead

There has been a somewhat less than reasonable reaction to problems of lead in the environment. Unfortunately, too many biologists have been undiscriminating with respect to the possible adverse effects of lead in water, soil, substrate, and air. There is no evidence that lead constitutes a health problem to fish in the United States. Few analytical data have been reported on concentrations of lead in fish in natural or experimental conditions. What data are available suggest that soluble lead is not present in natural waters of the United States in concentrations likely to be toxic to fish. There is no published evidence of any trend toward increased concentrations of soluble lead in natural waters. Much of the man-dispersed lead that is eventually washed into natural waters is probably precipitated owing to the presence of carbonates, hydroxides, and organic ligands in the water and settles to the bottom. There is no evidence that lead precipitated on the bottom of natural waterways is harmful to fish (1).

Finally, the basic phenomena of antagonism and synergism in biologically active chemicals must be recognized as applying to these regional waters.

A series of analyses of waters from the Colorado River and tributaries during 1970-71 gave values for lead which were always less than 0.01 ppm. It has been suggested that "from the data available, it seems likely that the global mean lead content for lakes and rivers lies between 1 and 10 ppb" (14).

In natural waters generally, the pH value is somewhere between 6.5 and 8.0. In the waters we have studied in the Rocky Mountain region, the values hover around neutrality, pH 6.8 - 7.2.

Secondly, the concentration of elements is usually low, 10^{-6}M. Finally, pure solutions of single elements are not found in nature. Wherever dissolved CO_2 is found, as in Rocky Mountain region waters, carbonates of lead precipitate. Hence, many of the laboratory exercises (where CO_2 is carefully excluded) dealing with the behavior of lead in solution are not pertinent to the conditions in natural waters.

A short reference to technical methods is in order. Atomic absorption spectrophotometry is the method of choice for lead in water because of its relative simplicity. The colorimetric method is more sensitive--but also more tedious. In our experience, the AA method is accurate within its limitations if one acknowledges the fact of interfering substances, notably calcium. False high lead values are found in samples that contain any appreciable amounts of calcium. The use of the hydrogen lamp blank to correct for calcium is routine in our laboratory. Random and unaccounted "spiking" of unknowns and standards is a part of our standard operating procedure. If these precautions are recognized, acceptable and useful data on lead in water may be obtained.

Lead profile studies in the substrate of high lakes (10,000 feet or higher) in the Rocky Mountains are underway. One example from Nymph Lake may be illustrative. A core 6.5 feet deep was taken. Lead content at different depths is given in the following table:

Depth	Average Lead µg/gm	Range µg/gm
5 - 6.5	8	4-12 (8)
3.5 - 5	10	6-18 (12)
2 - 3.5	16	8-20 (12)
1 - 2	21	9-28 (19)
0 - 1	25	14-34 (20)

Several points are of interest. Lead concentration has increased from an average of 8 µg/gm in the older or deeper layers to an average of 25 µg/gm in the younger or more superficial layers--a threefold increase during the time span. Moreover, the range of values is greater (20 µg/gm) in the younger layers than in the oldest (8 µg/gm); the difference in range is 2.5-fold. One may speculate on the reason for these values.

Aluminum

A number of fossil fuels may have associated with them aluminum salts. For example, oil shale contains significant amounts of dawsonite, sodium aluminum dihydroxy carbonate, $Na\ Al\ (OH)_2\ CO_3$ (16). Toxic effects of aqueous aluminum complexes in neutral and basic media to rainbow trout fingerlings were investigated under constantly flowing, controlled conditions of concentration, pH, and temperature. Toxicities of various concentrations were highly pH-dependent. Dissolved concentrations over 1.5 ppm aluminum caused drastic physiological and behavioral aberrations as well as acute mortality. Toxic effects of suspended aluminum, while they are more noticeable, at lower concentrations, are not as concentration-dependent as those of dissolved forms. The safe concentration of either dissolved or suspended aluminum is well below 0.5 ppm. Mortalities among test animals ceased almost immediately after aluminum exposures were concluded. Recovery times for fish exposed to the various tests were proportional to the severity of the test conditions and to the length of exposure. Normal growth resumed almost immediately in fish from the less severe tests, and after a period of a few weeks in fish from the more severe tests. After up to four months recovery, the average weight of fish surviving both chronically and acutely toxic aluminum concentrations is markedly below control average (8).

In Colorado, Utah, and Wyoming, extensive deposits of oil shale are found. Dawsonite is present in the spent shale which must be dumped in a very finely divided form. Aluminum salts leaching from such a dump into useful trout streams would be cause for concern. It is doubtful that human health would be in any manner threatened by aluminum salts in solution.

Mercury

The information available on mercury is inadequate to get a clear picture of the geochemical cycle of mercury or even to make accurate estimates of its abundance in common rock types. Data on mercury in coal and petroleum are scanty. Available data on mercury in natural waters indicate that most waters contain a tenth of a part per billion to a few parts per billion. No data are available to permit an assessment of contamination. Unpolluted air contains about 1 to 10 ng Hg/m^3. Natural pollution caused by volatility of mercury from ore deposits of mercury or base metals gave values up to 62 ng/m^3. Industrial pollution results from the burning of coal and perhaps petroleum and from metal smelters. No data are available on amount of mercury discharged or on its time of residence in the atmosphere (7).

Most samples of water from the Rocky Mountain region that we have analyzed for mercury have less than 10 ppb. Values above that suggest a pollution source which may be a municipal sewage outfall or a natural source in the form of a mercury ore.

Mercury in the substratum is usually higher than in the waters. Algae growing in streams with no detectable mercury have measurable levels probably as a result of a natural process of concentration.

One sewage outfall in western Colorado gave 0.07 ppm mercury.

It is claimed that dilute thermal springs contain readily detectable mercury. Sediments associated with some of these springs are rich in mercury containing about 50 to 5,000 times the mercury content of ordinary rocks, and the mercury contained is presumed to have been transported by the spring water. Petroleum and especially the tary residues of petroleum contain the highest determined mercury contents. Data in the literature for hot springs and volcanic gases are incomplete (17).

Our analyses of waters (but not substrates) from sulfur springs in western Colorado reveal no dissolved mercury.

The question has arisen a number of times whether there is a difference in the amount of mercury found in eutrophic lakes as compared with the so-called oligotrophic lakes. Mercury analyses demonstrated that 59 rainbow trout taken from an oligotrophic lake contained an average total mercury concentration of 0.17 ppm with a standard deviation of ±0.1 ppm and a 99% confidence interval of 0.14 to 0.20 ppm. On the other hand, 100 rainbow trout taken from a eutrophic lake had an average total mercury concentration of 0.07 ppm with a standard deviation of ±0.05 ppm and a 99% confidence interval of 0.06 to 0.08 ppm. The total mercury content of the soil in the vicinity of the two lakes is similar. However, the sediments of the eutrophic lake contained significantly higher levels of mercury than the sediments of the oligotrophic lake. Water samples taken from both lakes at various depths showed mercury levels of less than 0.1 ppb, the limit of sensitivity of the analytical method that was used. The removal of mercury by adsorption onto organic particulate matter probably is a key factor in the different mercury levels of fish in the two lakes. It is this adsorption mechanism that makes the mercury from pollution thus available for accumulation by fish that feed in the water column. Therefore, the persistence of mercury compounds in these two isolated lakes appears to be related to the organic character of each lake. Accordingly, mercury pollution appears to pose a greater environmental hazard in oligotrophic than in eutrophic lakes (3).

This fact might very well explain some of the anomalous results which have been reported from certain lakes in the United States with respect to the amount of mercury found in fish in these lakes in which the water showed no unusual quantities of mercury.

The fate of dissolved mercury is not always clear. Mercuric ion has been shown to be rapidly absorbed from water by goldfish. The extent of absorption is dependent on mercury concentration and time of exposure. This type of relationship is not unusual for almost any kind of soluble toxicant. As has been previously shown with other metals, mercury is known to concentrate initially in the external mucus secreted by the fish. The presence of mercury in water appeared to stimulate the secretion of mucus. It may be that increased volumes of mucus produced by the fish function as a protective mechanism for the fish against rapid harm resulting from dissolved mercury (15).

The concern which has been expressed in some quarters over the mobilization of mercury from sediments into aquatic vertebrates may require some modification. A recent study has been made of the aerobic mobilization of mercury from aquatic sediments into fish. It was demonstrated that mercuric sulfide in sediment is very slowly mobilized and picked up by fish (9).

The relation between methyl mercury concentrations in the liver and muscle of fish indicates the relation between accumulation and excretion rates of methyl mercury. The total mercury content of bottom dwelling aquatic animals is of little interest when one calculates the rate of mercury transport from bottom dwelling animals to the fish that feed on them. This situation obtains because the percentage of methyl mercury in the total mercury content of the bottom is generally low, specifically much lower than the percentage in fish, and the total mercury content is quite variable. The methyl mercury transportation from bottom dwelling organisms to bottom feeding fish is clearly small. By comparing the total mercury content of 3 ecological subgroups of benthic or bottom dwelling animals, it seems possible to separate situations where release of mercury prevails from situations where it has ceased (11).

Mercury estimations have been made in fish from various parts of the United States. For example, estimations were made on fish filet samples from throughout Wisconsin and from the boundary waters of Wisconsin in Lake Michigan, Green Bay, Lake Superior, and the Mississippi River. All fish from Wisconsin that were analysed contained some mercury; range of values was from 0.01 to 0.60 ppm; the average was 0.19 ppm. The highest mercury levels averaged 0.80 ppm; range of these highest values was from 0.06 to 4.62 ppm; these high values occurred in fish taken from below paper mills and from below a mercury cell chlor-alkali plant. Different species of fish vary in mercury content; the larger fish within a species often contain higher concentrations of mercury than do smaller fish of the same species taken from the same water. Walleye, sucker, redhorse, crappie, and bullhead frequently show the higher concentrations of mercury; pan fishes show lower concentrations. It is obvious that the levels of mercury in all important fish species must be estimated before the potential pollution problem can be evaluated adequately (12).

Water analyses by themselves are not adequate signals of elevated mercury levels. Biological concentrators must be included in a thorough study of any given aquatic ecosystem.

An example of concentration of mercury by aquatic organisms is provided by a series of studies we made on rainbow trout. The water showed no detectable mercury. Large fish of the 12" class had values of mercury in the muscle of about 0.02 ppm. Most fish under 6" had no detectable mercury in the muscles; a few had values of 0.003 ppm or less.

Mechanism of Action

The mechanism of action by which various trace metals exert their deleterious effects on aquatic organisms is not clear. A few *in vitro* studies have thrown some light on how certain metals may exert their toxic action (2). Relative changes in the activity of glutamic oxalacetic transaminase and lactic dehydrogenase in the blood plasma from white suckers were estimated after incubation with 49 compounds. These agents were principally inorganic chlorides at concentrations of the ions up to 2 mg/ml in the reaction mixture. A sequence of inhibitory effects was arranged for each enzyme. Dose-response curves were qualitatively similar for most of the chemicals. The glutamic oxalacetic transaminase was most sensitive to silver and mercury; the lactic dehydrogenase to palladium and mercury. Both enzymes were highly inhibited by metals which are highly toxic to aquatic animals. Correlations were studied between the inhibitory effect and certain physiochemical properties of chemicals; the best correlation was found between the inhibition of the glutamic oxalacetic transaminase and the equilibrium constants of metal sulfides.

The effect of some trace metals in the aquatic environment may be very subtle. A new possibly primary effect of heavy metals on fishes has been demonstrated. Heavy metals have been observed to affect fishes so as to cause a drop in the salt concentration in the blood serum. This change in the ionic content of the blood can cause the fish to become weakened and pronounced change can be fatal. Salt changes of zinc and copper in the blood have been monitored by osmometry and show the adverse hematological effects (13).

Dissolved Oxygen

Various studies have been aimed at artificial reaeration of inadequately aerated streams and lakes. At Colorado State University, a successful pilot study was carried out using Parvin Lake as the experimental body of water which was aerated in order to increase the total dissolved oxygen. Studies have been made of recent activities in river and stream aeration by artificial techniques; it seems that a workable engineering methodology is being developed for future river and stream aeration projects. The engineering developments we now see being implemented are based on a thorough review of the oxygen dynamics in rivers and streams and the capabilities from the engineering point of view of aeration systems. When one approaches the problem from a theoretical point of view, it becomes evident that aeration can be used only as a "polishing" action. It is naive to suggest that reaeration of a stream or river will solve all the problems of chemical overdoses. Such an expectation is neither sound nor attainable. Careful studies, based on theoretical considerations compared with actual measurements made in streams and rivers, indicate that artificial respiration or aeration can be applied successfully to raise dissolved oxygen levels to 5 ppm by the use of mechanical surface aerators, diffusers, downflow contactors, and side stream mixing (10).

This kind of treatment may find extensive use in the Rocky Mountain region for impoundments and some natural lakes. In our experience, the Rocky Mountain streams and rivers are well supplied with oxygen. Even when temperatures are fairly warm in these running waters, it is rare to find dissolved oxygen levels below 7 or 8 ppm. As a general rule, it is fair to say that dissolved oxygen is not a limiting factor in Rocky Mountain running waters.

Dissolved Oxygen Requirements

It is difficult to arrive at a scientifically sound and practically feasible conclusion with respect to the minimum dissolved oxygen concentration in which fish can thrive. It is obvious from a study of the literature that endurance limits have been established in the laboratory; generally these values are probably either lower or higher than the so-called true threshold values of tolerance in nature. A number of threshold values for dissolved oxygen have been reported in the literature. They vary widely. A major source of variation stems from different experimental methods used. An examination of available data indicates clearly that many of these methods are not reliable.

Interspecific differences and differences among individual fishes within a given species are rather great with respect to sensitivity to dissolved oxygen levels. A recent report maintains (4) that "reports of fully developed freshwater fish being killed within a day or two by reduction of O_2 concentration to levels above 3.0 mg/l in water of otherwise favorable quality are unusual and should all be regarded with some suspicion. They cannot now be accepted as convincing evidence that reduced concentrations not below 3.0 mg/l are intolerable for some fish under ordinary conditions in nature."

In some publications, it has been suggested that high concentrations of free dissolved carbon dioxide may aggravate oxygen deficiencies. A comprehensive evaluation of the available literature, however, suggests that high concentrations of free carbon dioxide probably will have virtually no effect on the response of fish to oxygen deficiency especially if the fish are accustomed to that level of carbon dioxide concentration. On the other hand, if the exposure to a slight increase in dissolved free CO_2 is sudden in the face of reduced oxygen levels, this combination is often lethal to fish. From the physiological point of view, it is obvious that the dissolved oxygen demand of a fish increases as the amount of dissolved free carbon dioxide in the water increases. As pointed out previously, fish rapidly adjust themselves to high concentrations of dissolved carbon dioxide. In nature, this fairly rapid adjustment to elevated carbon dioxide concentration in water will occur sooner than the reduction of oxygen to critical levels. The available evidence indicates that the effects of free carbon dioxide dissolved in water are not related to the changes in pH value of that water which result from the dissolved CO_2.

There are intraspecific differences in tolerance levels for dissolved oxygen in water. These differences seem to be related to geographical location. However, the mechanism of tolerance development and the permanence of any such developed tolerance is obscure at this time.

Some investigators have reported that super saturation of water with oxygen is lethal to fish. Other investigators find that there is no evidence for this conclusion. It is known that the excessive production of oxygen by aquatic plants during photosynthesis can at times cause fatal gas bubble disease in fish.

Fecundity of fish is reduced or even completely inhibited by oxygen embryos deficiencies. The development of fish embryos can be retarded. The size of the fish at time of hatching can be reduced and hatching can be delayed in the presence of reduced oxygen in water.

When all the literature available on oxygen requirements of fish has been reviewed, it is evident that the embryonic and developmental stages are more susceptible to oxygen deficiency than are the adult stages. Moreover, it is evident that the intraspecific variations are enormous--a fact which makes it virtually impossible to establish any one critical threshold level for dissolved oxygen. A quotation by Doudoroff and Shumway (4) is pertinent: "The widely accepted conclusion of Ellis (1937) that good, mixed fish faunas do not occur in waters in which O_2 falls below 4 or 5 mg/l is based on unreliable evidence and is contradicted by more reliable observations. Large numbers of fish species, including game fishes, have been collected in polluted waters where much lower concentrations were occurring regularly and even where concentrations not exceeding 4 mg/l apparently had persisted for a long time. Although some species may be eliminated, most warm water species evidently will continue to inhabit such O_2-deficient waters if the water quality is not otherwise too unfavorable."

It might be contended that the available fish food organisms necessary to support a healthy fish population might be oxygen dependent. It seems more likely, however, that the species of fish food organisms more tolerant of oxygen deficiency will increase in abundance in waters which are heavily enriched by organic material. The more sensitive species will disappear leaving less competition for the hardier species. Consequently, if the oxygen deficiency is not great enough to retard the growth of fish, one cannot conclude that the deficiency will impair the fish food resources. It seems probable that if the ambient water is not directly poisonous to fish and fish food organisms, the oxygen concentration itself will not be limiting for an abundant fish food organism population.

As a general evaluation, Doudoroff and Shumway (4) hold that:

> There is evidently no concentration level or percentage of saturation to which O_2 content of natural fresh waters can

> be reduced without causing or risking some adverse effects on the reproduction or growth and production of fishes inhabiting these waters. Yet, large reductions are not incompatible with the continued existence of some valuable fisheries.
>
> Water quality criteria on which regulatory standards designed for protection of fisheries in waters receiving wastes are to be based cannot be properly formulated without reference to the pertinent natural characteristics or the conditions of the waters and to desired levels of protections of fisheries. These levels of protection must be determined on the basis of socio-economic considerations. Attention to differences of waters and natural properties, such as O_2, that vary over a wide range, is essential because of associated differences of fish faunas inhabiting the waters and differences in natural productivity of the waters.

The use of kill-no-kill data to establish acceptable concentrations of oxygen for fishes is unwarranted. It is important to use some index or series of indexes more sensitive than mere death of the adult fish. Such criteria as incipient lethal or minimal oxygen concentrations tolerated indefinitely by 50 percent of the fish tested are critera of questionable value which in the past have been estimated with uncertain reliability.

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NITRATES: HUMAN AND ANIMAL HEALTH

J. Osteryoung

The element nitrogen is an important part of the biocycle and is a substantial component in primary rocks. With the exception of nitrogen gas, N_2, all of its inorganic forms are labile with respect to oxidation and reduction in the environment and very soluble in water. The many physical, chemical, and biological sources and sinks of the various forms of nitrogen make it difficult to identify the source of any nitrogen compound and to estimate the nitrogen balance, even in a restricted area such as a small watershed. The best known aspect of nitrate pollution is the addition of undesirable amounts of nitrate to surface waters which accelerates the process of eutrophication. However, sufficient exposure of humans and other mammals to nitrate can cause methemoglobinemia and has been implicated in a variety of other health problems. This paper describes the sources and transport of nitrogen in the environment and discusses possible health effects of exposure to nitrates.

The Occurrence of Nitrogen in the Environment

Nitrogen occurs in many chemical forms in the environment. Table 1 shows the total amount of nitrogen in various natural reservoirs of this element (1). Although the largest amount of nitrogen is contained in the atmosphere, this source is relatively unimportant in most problems involving the nitrogen cycle because it consists of nitrogen gas (N_2), which is extremely inert to chemical reaction. In other words, the rates of oxidation or reduction of nitrogen gas are much slower than the rates of many other nitrogen species interconversions.

TABLE 1

Total Nitrogen in the Environment (g/cm^2)

Primary rock	32,000
Atmosphere	755
Fossil N. (sedimentary rocks)	70-110
Surficial deposits	2×10^{-3}

The most important characteristics of the element nitrogen are the variety of its chemical forms found in nature, the rate of conversion from one form to another, and the high solubility of many of these forms in water. Table 2 shows some compounds of nitrogen which are involved in the biocycle. Living matter contains nitrogen in complex molecules such as purines, proteins, porphyrins, and others. As indicated in Table 2, these compounds contain nitrogen in a reduced state, and most commonly in the most reduced state. The primary products of organic matter decay, hydrocyanic acid, urea, and amines and amino acids, are broken down further into ammonia or possible hydroxylamine, depending on the valence state of the precursor.

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TABLE 2

Some Forms of Nitrogen in the Environment

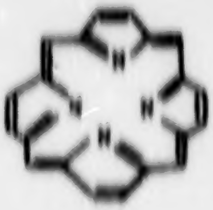
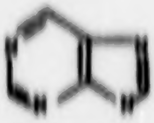
Name	Formula	Valence
Ammonia	NH_3	-III
Hydroxylamine	NH_2OH	-I
Nitrous acid	HNO_2	III
Nitrite	NO_2^-	
Nitric acid	HNO_3	V
Nitrate	NO_3^-	
Hydrocyanic acid	HCN	-III
Urea	$(NH_2)_2CO$	-III
Amines	$R-\underset{\displaystyle R''}{\underset{\vert}{N}}-R'$	-III
Porphyrins		-III
Proteins	-CONH-	-III
Purines		-I

Figure 1 shows a distribution diagram for the important inorganic forms of nitrogen in the environment. The diagram is drawn for a neutral water solution with total concentration of 10^{-3} M nitrogen. Also shown is the reducing power of the system as a function of the oxygen partial pressure in equilibrium with the water. The circled point corresponds to the oxygen partial pressure in the standard atmosphere. From this figure, we can see that under aerobic conditions the stable form of nitrogen is nitrate, that nitrite has a very narrow region of stability, and that systems have to be substantially anaerobic to contain ammonia (or ammonium ion) as the most stable form. Considering the equations for reduction of nitrate and nitrite to ammonia

$$NO_3^- + 9H^+ + 8e^- = NH_3 + 3H_2O$$

$$NO_2^- + 7H^+ + 6e^- = NH_3 + 2H_2O$$

it is apparent that the reduction takes place more readily in acid solution. Therefore, in solutions with pH < 7, the region of stability for NH_4^+ extends to more oxidizing potentials than it does in neutral solution.

In Figure 1, nitrogen gas is not included because of its chemical inertness. However, the other interconversions suggested are also slow in comparison with many common reactions in aqueous solution. In nature,

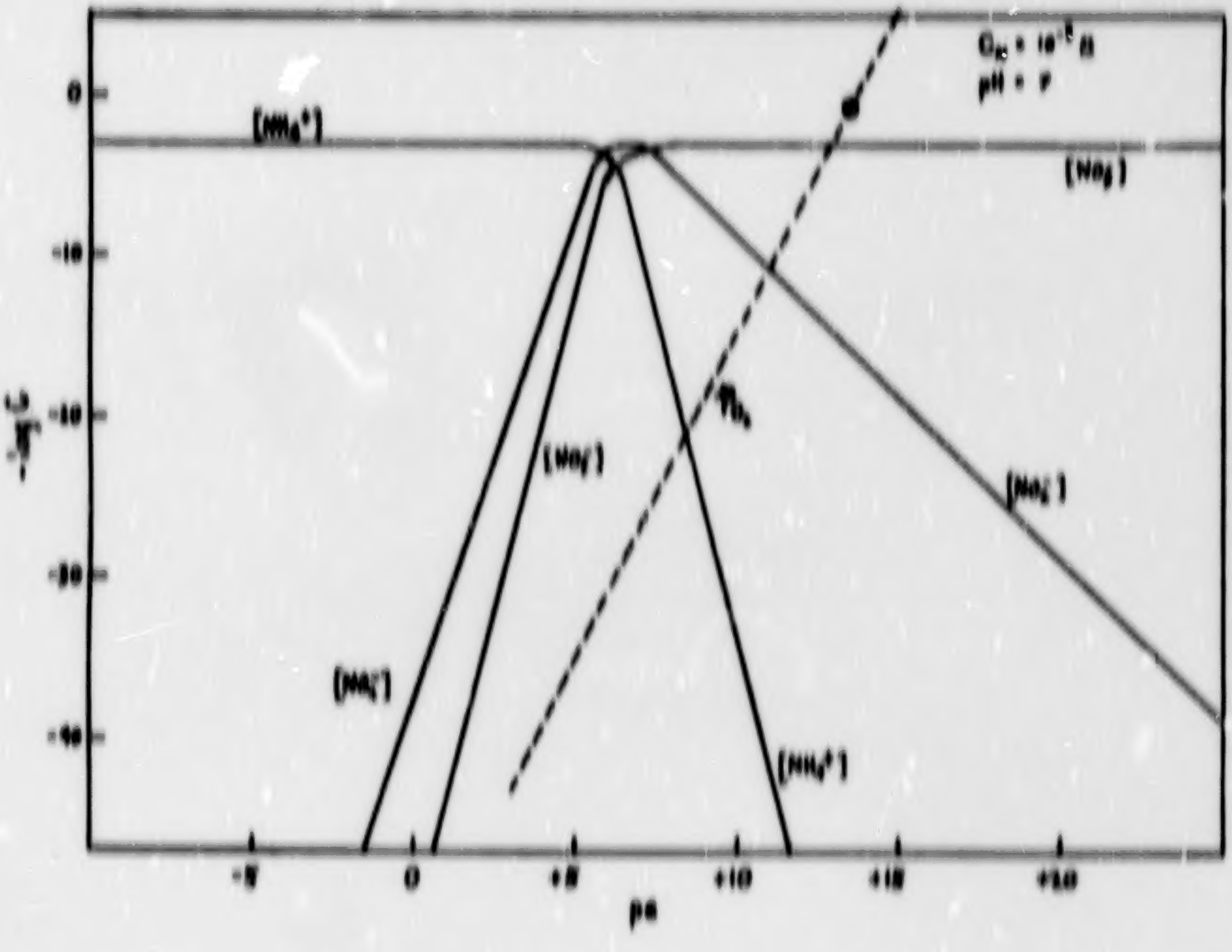


Figure 1. Distribution of inorganic nitrogen species as a function of oxidizing power and pH. pε = FE°/2.3RT, where F is the Faraday, R is the gas constant, T is the temperature in °K, and E° is the reduction potential vs. the standard hydrogen electrode.

interconversions of ammonia, nitrite, and nitrate are typically mediated or catalysed by microorganisms, *nitrosomonas* being the mediator for the ammonia/nitrite conversion and *nitrobacter* for the nitrite/nitrate conversion. It should be emphasized that the rate of the uncatalysed oxidation or reduction of any of these species to another is quite slow, and that therefore biological processes dominate the rates of change of nitrogen containing systems under most conditions.

Ground Water Nitrate Levels

Ranges and typical levels of nitrogen in water and in rocks are shown in Table 3 (1). Both surface water and ground water usually have nitrate as the predominant form, and the concentrations in ground water are typically higher. Ground water in contact with rocks can leach nitrate and also accumulate ammonium ion through exchange of calcium, magnesium, or sodium ions in the water with ammonium ions in the rock (2). Potassium and ammonium ions react similarly with clay minerals, and therefore high potassium concentrations in water should compete with ammonium ions for fixation in minerals, and release fixed ammonium ions through an ion exchange mechanism. One might expect rather high concentrations of nitrate in ground waters in contact with peat or oil shale because of their very high nitrate content.

TABLE 3

Nitrogen Content (as nitrate, ppm)

Surface Water	0-100
Ground Water	0-1,000
Igneous Rocks	$\sim$ 200
Sedimentary Rocks	$\sim$ 2,200
Peat	$\sim$ 70,000
"Unpolluted" Water	5-10

In soils almost all of the nitrogen is "fixed," that is, locked up in insoluble forms in clays or humic compounds; typically 0.1% or less is present as free nitrate or ammonium ions which can be leached into water and are available as plant food (2). For this reason, we focus our attention on nitrate and ammonium as sources of water pollution. The main sources of nitrates in water are natural aerosols (ammonium sulfate), precipitation, fixation, by microorganisms, organic matter, agricultural and industrial wastes, and rocks. The relative importance of these sources varies with region.

Table 4 summarizes data on the nitrogen content of precipitation (1). Although the amounts are small compared with those from sources such as fertilization, in grasslands or other relatively undisturbed environments they can be significant. Keeney and Gardner (3) point out that the amount of nitrogen required to bring a soil percolate to the 45 mg/l nitrate level is 2.27 lbs/acre-in. For example, if deep percolation out of the root zone is 6 in/yr, only 13.6 lb/acre/yr of nitrate would be required. This corresponds to 3.9 tons/mi^2/yr, which is in the normal range for nitrogen in precipitation.

TABLE 4

Nitrogen in Precipitation (Expressed as Nitrate)

NH_3 (mg/l)	∿ 1-2
NO_2^- + NO_3^- (mg/l)	∿ 0.2-1
NH_3 (tons/mi^2/yr)	0.5-14
NO_3^- (tons/mi^2/yr)	0.25-3
Total Nitrogen (tons/mi^2/yr)	0.7-16
(Fertilization (tons/mi^2/yr))	(100-1900)

Symbiotic nitrogen fixation through leguminous plants can be an important source of nitrogen in soils in intensively cropped areas. Legumes have been estimated to add in the range 50-250 ton/mi^2/yr nitrate, which can amount to a significant fraction of total soil nitrate added per year. Nonsymbiotic fixation is more difficult to estimate. The amounts of nitrogen fixed are much less than for symbiotic fixation, and it is not clear that existing data are applicable to real field situations. Total microbiological nitrogen fixation is undoubtedly an important source of soil nitrate in heavily farmed areas with extensive use of nitrogen fixing cover crops, but is unimportant in arid areas or areas of fallow soils.

The most important non-agricultural sources of nitrate pollution are domestic sewage and percolate from refuse (as from a sanitary landfill). The former has nitrate concentrations in the range 90-180 mg/l while the latter is in the neighborhood of 1300 mg/l. These can be extremely important sources of surface and ground water pollution in heavily populated areas and in arid or semi-arid areas. The typical "sanitary landfill," which is in practice usually a dump, should be suspected as a point source of nitrate pollution of ground water. The main nitrogen component of raw sewage is ammonia. Biological treatment oxidizes ammonia and organic forms of nitrogen to nitrate. The removal of nitrate (or ammonia) from wastewaters is under investigation, but no domestic sewage plants now have that capability (4-6).

Agricultural nitrate pollution is presently a much discussed and little understood subject (7). Commoner and others have taken the position that the nitrate burden in surface and groundwaters is due primarily to the heavy application of inorganic nitrates common in modern farming (8). Some criticisms of this point of view simply state that it isn't necessarily so, and if it is so, it is unimportant in light of the task of growing food for the world's starving millions (9). Other more substantive reviews of this question have provided insight to the nitrogen balance problem under specific conditions but have concluded that it is unwise to generalize.

In studies of bromegrass in North Dakota, Power found 17% of applied nitrogen lost under dryland conditions and 23% lost under irrigated conditions (10). However, under the dryland conditions, nearly half of the applied nitrogen appeared as soluble nitrogen in the soil. With the same fertilizer application (180 lb/acre first year, 270 lb/acre second year), the irrigated bromegrass contained 70% of the applied nitrogen while the dryland bromegrass contained only 36%. The implication is that the dryland was overfertilized. Stewart et al. (11) studied the middle South Platte area of Colorado and found soil nitrate levels shown in Table 5. Deeply rooted high protein crops such as alfalfa deplete soil nitrate so that the level is even less than in native grassland. Also, the nitrate levels in dryland culture are much higher (probably due to low organic matter content) than previously thought, and therefore, nitrate leaching from these soils is undoubtedly significant. Finally, this work concluded that despite the much higher levels of nitrate near feedlots, the total area of irrigated farmland was sufficiently great that feedlot contribution to nitrate pollution of groundwater was less than that of irrigated farmland. This is in contrast to the opposite assumption made in the northern Missouri area (12).

TABLE 5

Average Total Nitrate, 0-20 ft, lb/acre

Alfalfa	79
Native grassland	90
Cultivated dryland	261
Irrigated, not alfalfa	506
Corrals	1436

Stout and Burau (13), after extensive investigation of nitrate profiles in the Arroyo Grande basin, a heavily cropped area near San Luis Obispo, California, conclude that the primary source of nitrates to ground waters is tillage of native soils. The most important practical conclusion to be drawn from their work is that in areas such as San Luis Obispo with large populations and intensive farming, ground water levels of nitrate will prohibit the use of shallow aquifers for domestic water supply and either a surface water source or a primordial deeper aquifer must be used. Martin et al. concluded that farming practices in general rather than fertilizer application itself are most important in determining the extent of nitrate pollution (14). Prevention of erosion, minimum tillage (which reduces the extent of oxidation of organic matter to nitrate), and use of deep rooting cover crops such as alfalfa are suggested as farming practices which reduce the extent of nitrate loss. Smith takes a somewhat similar, if less constructive view, but he emphasizes the importance of domestic and animal wastes in rural groundwater (wellwater) pollution (12). Goldberg has summarized, but not evaluated, data on contributions of nitrate from various sources to water supplies (15).

The reason for controversy over the sources of nitrate pollution is that the reservoirs of nitrogen are large compared with small amounts of mobile nitrate; small differences in soil type, agricultural practices, etc. which slightly change rates of conversion from reservoir or fixed nitrogen to mobile nitrogen (mainly nitrate) make it nearly impossible to generalize the results of any observation or experiment. Commoner has attempted to solve this identity problem by recognizing that different sources of nitrogen may have slightly different isotopic nitrogen abundances, and therefore, the ultimate source of nitrate in water may be determined by its isotopic composition. This method was applied to the Sangamon River basin in central Illinois, and it was concluded from data obtained that fertilizer was the source of 50-60% of the nitrate in surface waters of the area (16). This approach has been criticized by Hauck *et al.* (17), and although the reply seems adequate (18), it is difficult for one not personally experienced in this area to judge.

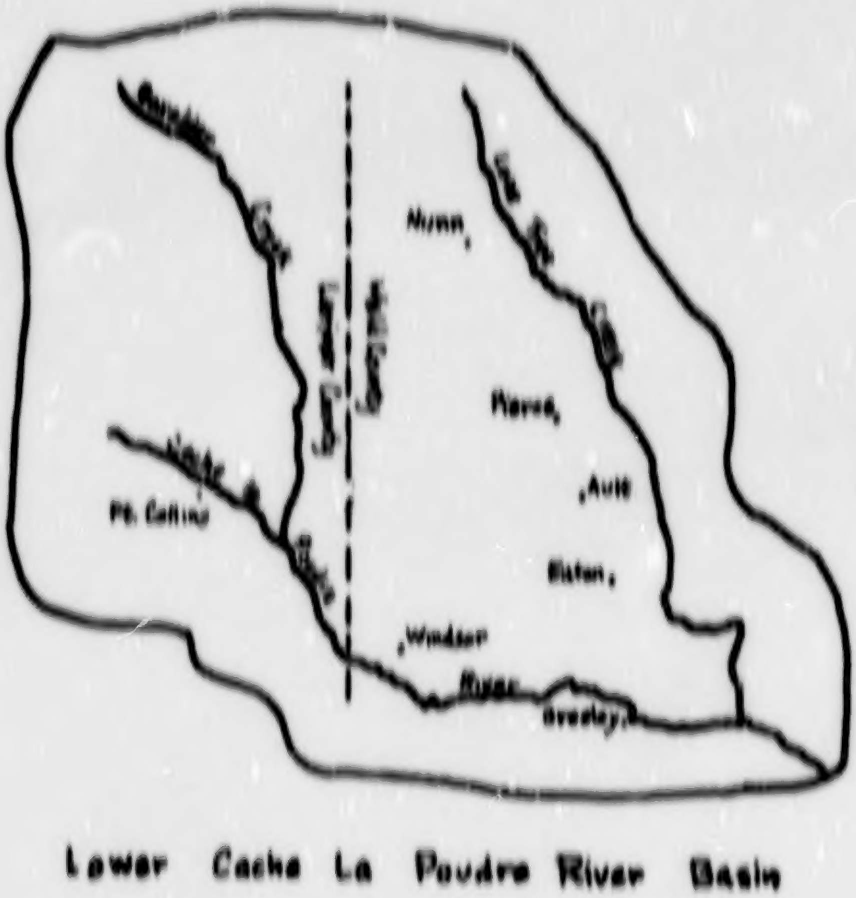


Figure 2. The Lower Cache La Poudre River Basin.

Assessing the importance of fertilizer runoff has specific implications for the environmental health specialist as well as the soil scientist and agriculturist. The Colorado River is an example of a multiple use surface water which is heavily polluted with nitrate and salts in general from its headwaters down, and is also used by large cities as domestic water supply. Agricultural practices in Colorado and other western states inevitably affect the quality of domestic water supplies in cities such as San Diego and Los Angeles. On a smaller scale, the severe pollution of family water supply wells, especially in Missouri, Minnesota, and Colorado has generally been attributed to local spot sources, usually feedlots or improper human waste disposal (14). Most studies have shown that lateral movement of nitrates from these sources is 300 feet or less, so that the source is isolated in the absence of high water table. However, it should be pointed out that there are numerous instances of new wells tapping high nitrate water in areas where there has been little prior human activity.

We have tried to determine the source of nitrates in areas in Colorado which have a long history of high nitrate levels in public water supplies (19). Figure 2 shows a portion of the lower Cache La Poudre River Basin near Fort Collins, Colorado. Fort Collins and Greeley obtain their municipal water from the foothills of the Rocky Mountains; this water is unusually pure. The other towns shown obtain water from local wells. Of these, Nunn, Pierce, and Eaton have rather high nitrate levels (30-160 mg/l) and Ault and Windsor very low levels. The town of Nunn, shown in Figure 3, is especially interesting because although nitrate levels there are consistently over 70 mg/l, there is no apparent source of nitrate pollution. Nunn has a population of 228, and it is isolated in the middle of a dryland wheat growing area where no fertilizer is used. The two city wells (1 and 2 in Figure 3) are drilled and properly sealed but shallow (39 and 52 feet, respectively) with water levels of 12 and 30 feet. Groundwater flow is NNW to SSE, and there is little human activity in the area NNW of the town. Fluorescein dye tests do not show direct contamination of drinking water wells by septic tanks in the area. All groundwater samples taken at sites shown in Figure 3 gave nitrate levels above 45 mg/l except No. 8, which is east of the Nunn groundwater flow pattern, and other sites, not shown in Figure 3, where a lower lying aquifer (≈ 200 feet) was sampled. This is an apparent case of "natural pollution" which has exposed the Nunn population to nitrate levels in public water as high as 160 mg/l for at least a decade.

Nitrate Content of Foods

The main sources of nitrate exposure for humans are water and foods. Foods fall into two categories, those with no deliberately added nitrates and those with nitrates (or nitrites) added during processing.

Lee (20) has recently reviewed some aspects of nitrate effects on human health and presents collections of data for nitrate and nitrite content of fresh and processed foods. Spinach, beets, rhubarb, lettuce, and cabbage have been found to have nitrate concentrations in the 0.1 - 0.5% range. Results of different investigators vary widely. This may be due in part to faulty analysis, but it can easily be accounted for by differences in fertilizer application, rainfall, etc. The natural accumulation

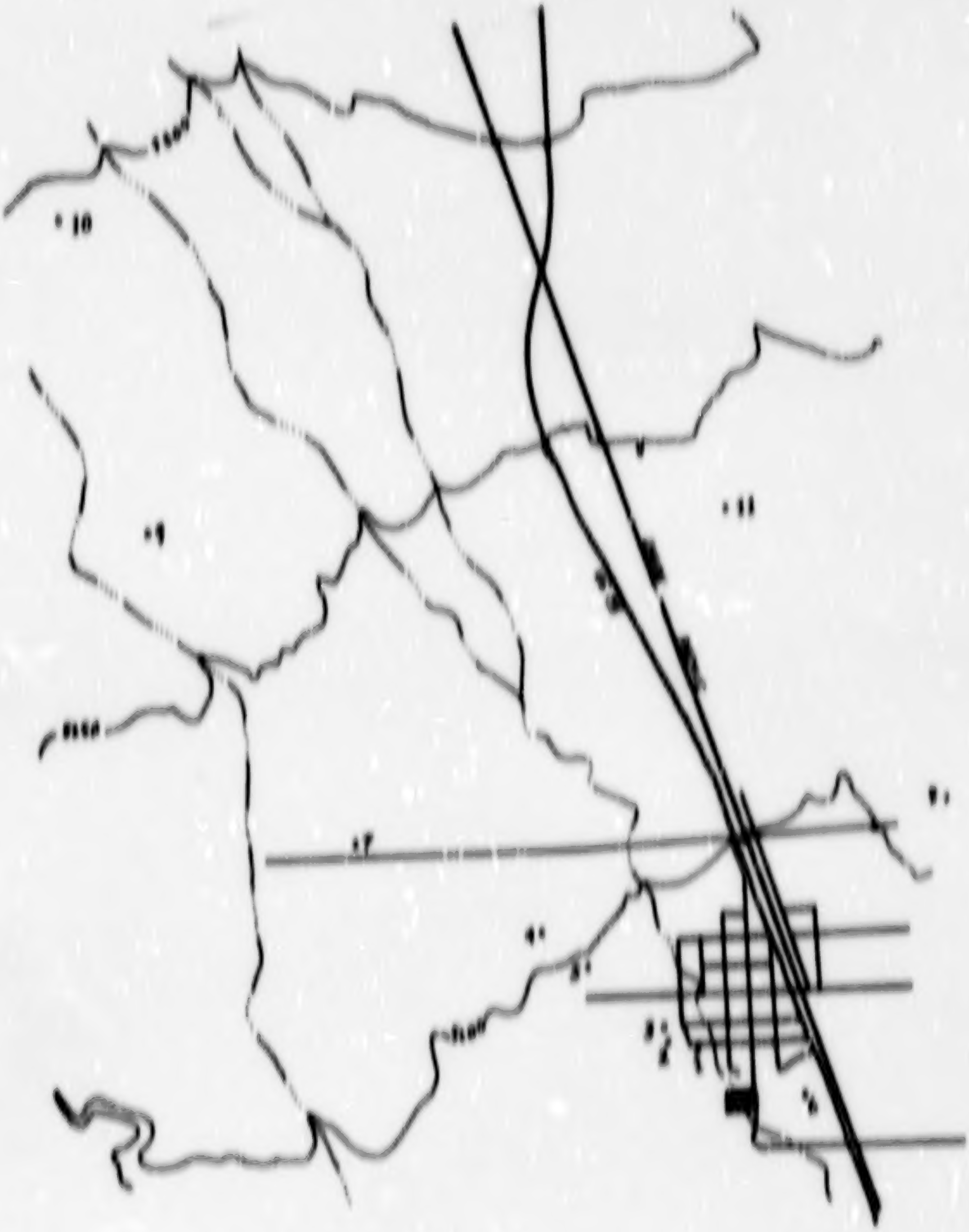


Figure 3. The town of Nunn, Colorado, showing groundwater sampling sites and drainage patterns to the north and west. No. 1 and 2, City of Nunn domestic water supply wells; ■ , city dump.

of nitrates in produce, particularly leafy vegetables, is highly dependent on agricultural practices and natural conditions. Fresh produce contains nitrate, but little or no nitrite. During storage, especially after processing, nitrate can be converted to nitrite. Storage above 15°C and anaerobic storage strongly favor production of nitrite. An extensive compilation of data on nitrate content of foods is contained in a recent NIEHS review article (21). Methods for nitrate determination are generally considered unsatisfactory, particularly in natural products and foods. Several recent analytical papers illustrate typical problems in analysis for nitrate or nitrite in cheese (22), milk and milk products (23), spinach (24), meat products (25), and baby foods (26,27).

Many processed foods contain nitrate or nitrite used as a preservative or for color enhancement. Section 121.1064 of Title 21 of the Code of Federal Regulations contains the following provisions regulating the use of sodium nitrite in foods:

1. As a preservative and color fixative in smoked, cured tuna fish products not exceeding 10 ppm in the finished product.
2. As a preservative and color fixative, with or without sodium nitrate, in smoked, cured sablefish, cured salmon, and smoked, cured shad not exceeding 200 ppm and the level of sodium nitrate not exceeding 500 ppm in the finished product.
3. As in (2), in meat-curing preparations for home curing of meat and meat products with directions for use meeting the specifications of (2).

Section 121.1230 specifies conditions for using sodium nitrite in combination with sodium chloride in the preparation of smoked chub (whitefish) to aid in inhibiting outgrowth and toxin formation from Clostridium botulinum type E. The sodium nitrite content of the edible portion of the finished product must be not less than 100 ppm and not greater than 200 ppm.

Under title 9, Section 318.7, sodium and potassium nitrite and sodium and potassium nitrate are authorized for use in cured meat products under conditions providing no more than 200 ppm nitrite (sodium nitrite) in the finished product.

In addition, baby foods are allowed to have no more than 25 ppm nitrite. Analyses carried out by the Consumer and Marketing Service, USDA, of a variety of baby foods containing eggs or meat showed nitrite as sodium nitrite in the range 0–28 ppm and nitrate as sodium nitrate in the range 0–175 ppm (28). It is interesting that with the exception of Gerber products, the manufacturer is not identified. In general, each sample had "none" or ≤ 13 ppm sodium nitrite and "none" or ≤ 20 ppm sodium nitrate. However, Lee (20) presents a summary of results for nitrate content of baby food vegetables by different workers, 1949–1969, which shows nitrate in the 1000 ppm range for many samples and substantial concentrations (say, >30 ppm) in most samples. The Joint FAO/WHO Expert Committee of Food Additives in 1962 recommended no acceptable dose level of nitrates and nitrites for infants below six months of age (29). A summary of the most recent report of this committee re-emphasized the importance of reducing the nitrate and nitrite intake of infants to a minimum (30).

The origin of standards for nitrates and nitrites in meats is obscure. Apparently the standard of less than 200 ppm sodium nitrite in the finished product resulted from experiments made by a meat processing company under USDA supervision in which it was found that cures with the desired properties (taste, color, etc.) could be obtained with nitrite treatment under conditions resulting in meeting this standard (31). That is, the standard was written to fit a rather limited set of experiments suited to the needs of the meat processing industry. Investigation in Britain in 1940 demonstrated that bacon, one of the worst offenders (as high as 0.27% nitrite after storage), can be prepared at the 10 ppm nitrite level with no impairment in the curing process (32). Although one must be realistic about quality control problems, present knowledge of the health effects of nitrates and nitrites would suggest the adoption of lower tolerances for these substances in foods. This is the general tone of a recent government report on nitrates and nitrites in foods (33). Reporting of the controversy surrounding the food additives problem has been evenhanded, even thoughtful, in scientific trade journals (34) but less accurate and more frivolous in corresponding medical journals (35).

Nitrate Toxicology

Those adverse health effects involving nitrate for which a mechanism is known or reasonably suspected involve, as a first step, the reduction of nitrate to nitrite. We shall first outline the known or suspected effects of nitrate and related compounds on human and animal health, and then discuss in more detail three problems deserving special attention, methemoglobinemia, chronic cardiovascular toxicity, and nitrosamine toxicity.

Organic and inorganic nitrates and nitrites have two primary pharmacological actions: the relaxation of smooth muscle, especially small blood vessels, and reaction with hemoglobin to form methemoglobin (36). Each compound differs from the rest in the relative efficiency of these two actions. For example, amyl nitrate is an effective vasodilator used for symptomatic relief from angina, while sodium nitrite (or nitrite ion) is especially effective in the formation of methemoglobin. Acute toxic reactions to nitrates are based on these two properties. Organic nitrates with high lipid solubility are readily absorbed through the skin or mucosa or, if the vapor pressure is sufficiently high, through the lungs. Water soluble inorganic nitrates or nitrites must be ingested (or injected) to produce toxicological effects. The metabolism of nitrates is not known, but inorganic nitrates or nitrites are rapidly absorbed in the upper intestine and excreted in substantial amounts.

Intoxications which primarily produce methemoglobinemia are characterized by acute cyanosis, nausea, vomiting, collapse, and so on. Cyanosis is clinically evident when the methemoglobin level reaches 10-15% of the total pigment circulating in the blood; levels of 60% produce stupor while the lethal range is probably 70-85%. There is a reasonably good correlation between the severity of the intoxication and the methemoglobin level. However, some cases of cyanosis have been reported with methemoglobin levels as low as 3% (37). Infants are especially at risk, because of low gastric acidity which promotes nitrite formation in vivo from ingested nitrate, and because of the susceptibility of fetal hemoglobin to methemoglobin formation.

The standard treatment for methemoglobinemia consists of methylene blue injections; the mode of action is not understood, but the treatment is universally successful. Although ascorbic acid is effective in reducing methemoglobin concentrations, its use is not appropriate in the case of acute intoxication. The use of nitrite as treatment in cases of acute cyanide poisoning has been substantially discredited.

Intoxications which primarily cause smooth muscle relaxation produce cardiovascular and central nervous system reactions. Initial shock is produced by dilation of postarteriolar vessels; this vasodilation is not blocked by any recognized drug, and in fact arteriolar constrictors probably should be prohibited in treatment of the condition. Increased intracranial pressure and cerebral anoxia are implicated in nervous symptoms. Circulatory inadequacy is complicated by methemoglobinemia, resulting in cyanosis and anoxia. With exception of treatment for secondary methemoglobinemia, supportive symptomatic treatment, including transfusion, is all that is available.

There are numerous reports in the literature of acute nitrate poisoning, often resulting in death, due to deliberate or accidental ingestion of drugs such as amyl nitrite or nitroglycerine, ingestion or superficial contact with various dyes and polishes containing organic nitro compounds, consumption of water high in nitrate (in infants), and ingestion of processed foods not meeting U.S. government regulations. One particularly striking case involves acute nitrite poisoning with severe cyanosis in a healthy adult patient normal in methemoglobin reductase activity; the poisoning occurred from eating sausage well within the standards (180 ppm sodium nitrite) (38). Because conditions in the rumen are especially conducive to production of nitrite from nitrate, cattle, sheep, etc. are especially susceptible to nitrate poisoning (39).

Nitrates apparently also interact with vitamins or vitamin metabolism. High nitrite exposure causes Vitamin A deficiency; evidence suggests on the one hand that this is due to impaired thyroid function which inhibits carotene transformation to Vitamin A (40) and on the other that it is due to direct destruction of carotene and Vitamin A in the intestinal tract (41). Significantly higher levels of methemoglobin are produced by nitrite in animals exhibiting Vitamin C deficiency than in those with normal Vitamin C levels (42). Vitamin C is capable of reducing methemoglobin and is used in management of hereditary methemoglobinemia (36).

Epstein has reported on the involvement of sodium nitrate in a case of palindromic rheumatism (43).

Methemoglobinemia. The relations of nitrite and nitrate consumption to clinical methemoglobinemia have been comprehensively reviewed by Lee (20). The etiology, diagnosis, and treatment of this condition as described above is well established. Although some populations with impaired methemoglobin reductase activity, pregnant women, and people stressed by starvation are at greater risk from nitrate exposure than the general population, the highest risk group is infants under 9 months. In most cases, the major exposure to nitrate in this age range is from water; this exposure can easily be controlled. Therefore, public health officials have a special responsibility to prevent this condition by finding if nitrate levels in local water supplies are dangerously high and publicizing this problem if it exists.

Infant methemoglobinemia is relatively well-known in the midwestern United States and most of the reported cases are from this area. However, failure to report or incorrect diagnosis of mild methemoglobinemia may result in substantial underestimate of the prevalence of the condition. Methemoglobinemia in infants is often associated with gastroenteric infection, probably because impaired adsorption of nitrate in the intestine increases the rate of conversion of nitrate to nitrite. Diarrhea has been reported as an effect of nitrites (20). Change in regimen as treatment for diarrheal diseases might often remove the source of nitrates and automatically reduce blood methemoglobin levels. Adequate awareness of this problem on the part of public health professionals should eliminate mortality and substantially reduce morbidity due to elevated blood methemoglobin.

The effects of subclinical methemoglobinemia or chronic elevation of methemoglobin levels above the normal range are unknown. Kohl *et al.* are now attempting to determine normal values for methemoglobin in blood and to relate methemoglobin levels to nitrate consumption and biochemical parameters of blood (44). There is reason to suspect that elevated methemoglobin levels enhance atherogenesis in the rat (45). It is known that chronic exposure to carbon monoxide with resulting high levels of blood carboxyhemoglobin accelerates the development of atherosclerosis in rabbits and squirrel monkeys (46,47). The effects of carbon monoxide are also more severe at higher elevations. One might expect elevated methemoglobin levels to exhibit the same characteristics. These facts taken together show the importance of discovering the relation between nitrate consumption and methemoglobin levels and of carefully studying the health effects of elevated levels. The physiological connection with atherosclerosis also has a bearing on the epidemiological data of the following section.

Hypertension and Sudden Death. Morton has investigated the relations among drinking water constituents, hypertension, and chronic cardiovascular disease in Colorado (48). He found no correlation between chronic cardiovascular disease mortality (ischemic heart disease, vascular lesions of the CNS, and general arteriosclerotic and peripheral vascular disease) and altitude or geographic area. However, both hypertensive heart disease mortality and hypertension prevalence were significantly greater at lower altitude. In addition, hypertension mortality and prevalence were higher in river basins with greater water hardness and with higher nitrate concentration. Although the use of mean nitrate values in public water supplies as an index of exposure of the sample population is not very satisfactory, criticisms of this study all suggest that the correlation between nitrate exposure and hypertensive disease is underestimated.

The hypotensive effect of organic nitrates is well known and has been suggested as the cause of elevated diastolic pressure among workers in the explosives industry. Morton has reviewed the industrial toxicological problem of the explosives industry (49). "Monday morning angina" including sudden death has clearly been established as an occupational hazard of explosives workers.

Schmidt and Knotek have examined the incidence of infant methemoglobinemia in Czechoslovakia and suggest nitrate poisoning may be responsible for 10-20% of the cases of sudden death in infants up to three months of age (50). The sudden infant death syndrome (crib death) is poorly understood, and furthermore, epidemiological study of this syndrome is difficult because of problems in determining cause of death and fads in reporting. This makes it hard to obtain reliable mortality data. In spite of pessimistic views of further possible contributions of epidemiology to the understanding of this syndrome, several factors suggest that the role of nitrate exposure should be explored (51). The wide range of methemoglobin levels associated with onset of clinical methemoglobinemia (3-15%) shows individual variation which might also be prevalent at lower levels. Therefore, some infants, particularly premature and low birth weight infants, might experience hypoxia at methemoglobin levels generally considered normal. These groups have a higher incidence of crib death. Studies in Ontario, Canada have shown that 85% of verified cases of crib death are of infants exclusively bottle-fed. These data led to the hypothesis that hypersensitivity to cow's milk is a contributing factor. However, the hyperallergenic hypothesis has not been substantiated, and association of crib death with bottle-feeding might also implicate the water used in formula preparation. Crib death occurs at rates of 2-3 per 1,000 in the same age cohort that infant methemoglobinemia is prevalent, and it accounts for about 20% of all infant mortality. We have not found any reports which have examined the hypothesis that nitrate exposure is a contributory factor in crib death, but we feel such a study would be valuable.

Nitroso Compounds. The general and acute toxicity, teratogenicity, mutagenicity, and carcinogenicity of a variety of nitrosamine compounds in mammals is well established (52,53,33). Experiments show that about three-fourths of the nitrosamines tested produced lesions in test animals. Dose-response relationships, no-effect levels, limits of toxicity, and margins of safety are unknown. The specificity of carcinogenic activity of various nitrosamines provides a basis for epidemiological studies in humans. Recently, dimethylnitrosamine (DMNA) in an alcoholic beverage called Kachasu has been implicated in the high incidence of esophageal cancer in East Africa (54,55).

There is no question that the nitrosamines are potent carcinogens which can be dangerous to human health. In addition, they have the remarkable property that a single dose can induce cancer (56). However, human exposure and the relation of exposure to nitrate and nitrite exposure are not presently known. Major areas of concern are the presence of nitrosamines in foods, especially cured meats and fish, and *in vivo* synthesis of nitrosamines from nitrates and naturally occurring amines or drugs.

Nitrosamines are compounds of the form RR'N-NO where R and R' represent some organic moiety. They can form by reaction of nitrite and various secondary amines, quaternary ammonium ions, urea, etc. Therefore, the occurrence of nitrosamines in foods or the production of nitrosamines *in vivo* depends on the presence of both nitrite and a suitable nitrogen-containing substrate under chemical or microbial conditions which facilitate the reaction. Trimethylamine oxide is present in relatively large amounts in marine fish used to make fish meal to which nitrite is added as a preservative. Hepatotoxicity in sheep and mink has been caused by DMNA from this source (57-60). DMNA has also been found in cured meat and fish products (53,61-64). Various investigators have found that nitrosamines can be formed *in vivo* or under physiologic conditions *in vitro* (65-67). Lijinsky has suggested that many common drugs which are secondary amines may react *in vivo* to produce carcinogenic nitrosamines (68). It has also been suggested that the heavy losses of livers of feedlot cattle is due to formation of nitrosamines from nitrates and amines present in feeds (69).

Investigation of this problem is severely hampered by the lack of adequate analytical methodology. There are a wide variety of nitrosamines, they are in general labile compounds which are easily formed or destroyed by sample treatment, and there are many interferences in the usual analytical procedure. The most exacting digestion-distillation-extraction-GLC-MS techniques give apparently reliable identification and quantitation but are far too expensive for routine analysis required in survey or monitoring programs. All phases of investigation of the nitrosamine problem urgently require more reliable and less expensive analytical methods. This subject is discussed in several recent articles and reports (53,67, 70,71).

The presence of nitrosamines in the human environment has been called a menace and a possible human health hazard (72,73). The latter view seems the balanced one at this point. The known potent toxic effects of these compounds and their suspected presence in humans from ingestion or synthesis *in vivo* argues first that research on this problem should proceed in all fields at a fast if not urgent pace, and second that human intake of the known precursors of nitrosamines, especially nitrite, should be held to levels as low as practical.

Exposure to Nitrate

Estimation of individual exposure to nitrate is important in any epidemiological study and in the setting of standards. Van Camp has reviewed the process by which PHS set the drinking water standard at 45 mg/l nitrate (10 mg/l nitrogen) and concluded that it is based primarily on retrospective studies of incidence of infant methemoglobinemia (74). There are two interesting papers which try to establish some theoretical range for an appropriate standard.

Burden has reviewed data on nitrate toxicity in adults and infants and in animals (75). He concludes that the lethal dose is about 20 mg/kg nitrite nitrogen or 66 mg/kg nitrite. This would correspond to about 90 mg/kg nitrate if there were complete conversion. He then assumes that the permissible dose is 0.2 times the lethal dose, or 4 mg/kg nitrite nitrogen. Assuming complete conversion, he concludes that permissible limits of nitrate nitrogen in water are 6 mg/l for infants in the tropics and 45 mg/l for adults in the tropics, or 24 mg/l and 240 mg/l in England. These figures are based upon estimates of water intake and calculated for 3 kg infant weight and 60 kg adult weight. The corresponding figures for nitrate concentrations are 27, 200, 106, and 1600 mg/l. Dry climates were not considered, nor was individual variation allowed for. Still, the lowest calculated level, 27 mg/l nitrate, is well below the current USPHS standard for nitrate.

Winton *et al.* (76) have calculated the hypothetical dose of nitrate required to convert 10% of hemoglobin to methemoglobin, assuming 80% bacteriological conversion of nitrate to nitrite. At 3 months for a 5.4 kg infant, this dose would be 9 mg/kg/da nitrate, or assuming consumption of one liter of water, 49 mg/l nitrate or 11 mg/l nitrate nitrogen. These numbers are very near the present USPHS standard of 45 mg/l nitrate (10 mg/l nitrate nitrogen).

These approaches, of course, do not allow for individual variation, and they are aimed primarily at preventing clinical infant methemoglobinemia. Further epidemiological study of the chronic effects of nitrate exposure must include estimates of individual nitrate exposure based upon nitrate levels in water, daily water intake, dietary and food handling habits, Vitamin C consumption, and so on. Infants are the group at greatest risk for developing methemoglobinemia, and probably also for health problems such as essential hypertension which might be associated with chronic exposure to nitrates. They also have a regimen that can expose them to much higher levels of nitrate than adults in the same household. Formula might be made from powdered milk substitute (Similac, etc.) using high nitrate water, while adults would get much of their liquid from canned or bottled beverages. Infants' solid food might consist of commercial baby foods which contain high nitrate and nitrite levels (ham and bacon are favorites) and of vegetables, some of which are especially sensitive to conversion of nitrate to nitrite during storage. Adults would normally have a smaller fraction of cured meats in their diet and fewer leftover vegetables. Finally, the first and most popular meat fed to infants is usually the ubiquitous hot dog. Lee's jocular comment that it is fortunate that most infants don't eat much corned beef (20) displays curious ignorance of this dietary vagary of the American public.

Summary and Conclusions

It is known that methemoglobinemia is a serious and often fatal result of nitrate exposure. On the basis of present evidence it is almost certain that some people are indirectly exposed to carcinogenic nitrosamines through nitrate or nitrite exposure. Chronic effects of nitrate exposure are unknown but could well include hypertension and atherosclerotic disease. There is some possibility of involvement of nitrate exposure with sudden death in infants and adults.

Water and foods both contain nitrates, often at levels which could be hazardous, especially to infants. In many cases, the source of nitrate is "natural" and difficult to control (in fresh spinach, for example, or a "virgin" water supply). In others, such as cured meat and fish products, nitrate (or nitrite) is deliberately added.

Both the hazards and the sources of nitrate exposure are complex. Realizing this complexity, we can nevertheless adopt policies which tend to minimize nitrate exposure, encourage public health officials to be alert to the dangers of high nitrate levels in drinking water supplies, and move forward on the epidemiological and physiological studies required to better assess the human and economic costs of nitrate exposure. At this stage, certainly all steps possible in the area of education should be taken, from requiring that methemoglobinemia be reported in disease statistics to providing proper instruction in home economics classes on preparation and storage of foods. It is imperative that the scientific investigation of this problem proceed vigorously to provide us with the definitive information required in the difficult political process of setting standards.

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AIR POLLUTION AND HEALTH

C. J. Love

Abstract

The best information available indicates that air pollution is a significant factor in the production and severity of illness. Deaths attributable to factors possibly related to pollution have increased significantly during the past several decades.

Disease or death occurs when environmental hostility overcomes the human defenses. This process can be described and the effects recorded.

Community studies conducted throughout the United States by the Environmental Protection Agency are documenting the associations of specific pollution levels on the frequency of acute and chronic respiratory illness as well as the irritation of symptoms in susceptible segments of the population, such as asthmatics, bronchitics, or elderly individuals with respiratory or cardiac illnesses. Recent results are reported.

Introduction

The subject of air pollution has become quite fashionable within the past several years; but in spite of the fad, there is little agreement about what should be controlled and the extent to which it should be controlled. Each individual's point of view is influenced or biased by his own position in life. Thus, we all want total elimination of pollution if someone else is going to pay for it; on the other hand, we are more inclined to think in terms of a reasonable level of control if the costs are to come directly from our own pocketbooks.

Eventually, however, whatever our own personal views of the subject, the question will arise about just how dangerous is air pollution; how many people are affected really; and how are adverse effects produced. To develop the answers to such questions let us consider the following few points, many of which were lifted or paraphrased from reports of Dr. Bertram Carnow, a well-known Chicago epidemiologist, and Dr. Patrick Lawther, a British epidemiologist.

Our country has the highest standard of living in the world. But our standard of health is not nearly as good. For instance, today, a 30-year-old American man can expect to live only about two and a half months longer than he did 30 years ago. In 16 other countries, people have greater longevity than we have.

The diseases that are killing people in this country are cardiovascular illnesses, bronchitis and emphysema, and lung cancer. Deaths from bronchitis and emphysema have increased tenfold since World War II. In a recent year, they accounted for close to 30,000 deaths as a primary cause, and nearly twice this many as an associated cause.

Bronchitis and emphysema are the second highest cause of disability in Social Security recipients under the age of 65, and they cost American taxpayers more than one hundred million dollars a year in payments. Also, emphysema and bronchitis patients have hospital stays almost twice as long as average.

Thirty years ago, lung cancer was a rare disease. Last year, it killed more than 50,000 men and over 10,000 women. It has been estimated that by 1980, lung cancer will kill at least a million people a year.

Thirty years ago, cardiovascular diseases accounted for some 20% of the deaths in this country. Last year, the figure was more than 50%. Cardiovascular diseases killed over a million people; coronary artery disease, usually in the form of heart attacks, killed 500,000. The diseases that are killing people, then, are in large measure, not bacterial or viral in origin; they seem to relate to events which began many years earlier; and they seem to have more than one cause.

Now, if we have diseases that are killing people and that have long incubation periods, it should be obvious that we had better begin doing something about it right now; but of equal importance is the fact that we must think in terms of prevention because many of these diseases have very high mortality rates once they emerge as distinct entities. For instance, almost half of the 500,000 deaths from heart attacks occurred during the first attack, about 30,000 during the first hour.

It is now almost certain that all of these diseases have a direct relationship to air pollution. There are other causes, to be sure. There is no question, for instance, that cigarette smoking is a major factor. But air pollution, too, has a significant role in the causation of these diseases.

Again referring to the reports of Dr. Carnow and Dr. Lawther, it is not hard to accept the fact that life is a continuous struggle to adapt to what is essentially a hostile environment--and our environment is hostile. We fight every day to breathe, to eat, to remove waste, and to adjust our temperature, chemistry, and body fluids. For this struggle, we are equipped, partly at birth and partly through development, with certain genetic, constitutional, social, and cultural defenses. If we are deprived of these defenses, host resistance drops. For example, cystic fibrosis and asthma seem to make people more susceptible to lung ailments, i.e., a child with either of these diseases is not equipped to adapt as easily to the stresses of the environment.

Disease or death can occur either when the environment overcomes an individual with very low resistance or when the environment becomes so hostile that it overcomes those with even relatively high resistance. The latter is what happened in London in 1952, when 4,000 people died in a pollution-heavy inversion and during other major air pollution episodes.

What is more important, however, is the chronic pollution or long-time exposure to less significant levels, which has a totally different effect. It is known from experience that the acute pollution produces death and worsening of disease. It is suspected very strongly that chronic pollution is responsible also for the production of disease. In general, it is believed to evolve in the following manner. If you irritate the lung chronically, it will produce more mucus than is necessary, and the glands will develop and become large and excrete masses of mucus which require coughing and spitting to remove. Patients with this condition are said to have bronchitis, i.e., the cough produces clear phlegm without infection. There is much evidence, epidemiological and experimental, to suggest that this picture is caused by cigarette smoking even more often than by air pollution. In the second stage of this disease, which occurs after repeated or continuous exposure, the secreted mucus becomes infected and then the patient begins to cough up green or yellow sputum. And eventually, the infection begins to destroy lung tissue and to produce emphysema. These changes are not reversible, and there is a great deal of evidence being collected to indicate that one of the important factors producing complicated chronic bronchitis is air pollution.

In addition, an enhancement of susceptibility to infection is associated with irritant air pollutants. The major protective mechanism of the tracheal-bronchial tree resides in the cilia, which beat several times a second and escalate material out. The normal mucus blanket traps impurities and bacteria, and these are then swept out by the cilia. But inhalation of relatively small doses of sulfur dioxide, nitrogen dioxide, ozone, or the smoke from cigarettes can cause transient paralysis of these cilia. Ultimately, after repeated or continuous stress, the cilia and the cells containing them can be destroyed, thus eliminating the defense and rendering the subject more susceptible to infection. The bronchial lining is replaced in patches by different cells, which can tolerate a lot of insult but have no ability to clear the lung. Besides, it is thought that these cells, called squamous cells, undergo degeneration and can ultimately become cancerous.

A further effect of air pollutants on respiratory defense mechanisms is the impairment of the activity of pulmonary macrophages. These are the cells deep in the lung, which engulf and remove foreign materials including bacteria and other particulate matter. Inhibition of this activity then increases susceptibility to infection and the potential for direct tissue irritation or damage in the lower recesses of the lung.

The net effects of major gaseous air pollutants, then, is to increase susceptibility to acute respiratory illnesses and to chronic respiratory illnesses such as emphysema and bronchitis, and to impair the oxygen transfer mechanism within the lungs, thus placing a greater burden on the heart to effectively deliver adequate quantities of oxygen throughout the body.

Epidemiological Studies

To illustrate the extent to which at least some of these conditions are being produced in this country, I would like to review very briefly our program of epidemiologic studies and the results of these studies obtained during the past two years.

CHESS, an acronym for Community Health and Environmental Surveillance System, is a national program of epidemiologic studies designed to measure simultaneously environmental quality and sensitive health indicators over time in sets of communities representing exposure gradients to common air pollutants. The purpose of the CHESS program is to evaluate existing environmental standards, to obtain health intelligence for new standards, and to document the health benefit of air pollution control.

CHESS Area Sets. A CHESS set consists of a group of three to seven communities selected to represent an exposure gradient for designated pollutants. Each community within a CHESS set is a middle class residential neighborhood containing three or four elementary schools (500 to 1000 children per school) and usually a junior and senior high school. The economic characteristics of neighborhoods are carefully matched within the same CHESS set.

Each CHESS set includes high, intermediate, and low exposure communities within which air quality varies from day to day. Health indicators are contrasted over time and space within this framework. Replication of pollutant exposures in several CHESS sets permits the development of exposure-effects models in one area and the confirmations of the models in another.

Health Indicators. CHESS health indicators focus on measures of selected acute and chronic diseases, altered physiology and pollutant burdens. These indicators may be conveniently divided into two categories: health effects attributable to short-term pollutant exposure and effects attributed to long-term exposure. Acute health effects are observed by following systematically, pre-enrolled panels of subjects, and comparing response frequency against daily variations in pollutant levels. Various time-series analyses are employed to isolate the effects of temperature and season on response frequency. Simultaneous observation in low exposure communities permits the quantitation of the effects of environmental covariates such as temperature and the detection of pollutant-temperature interactions. These health indicators are related to exposures of 1 to 96 hours, depending upon the nature of the response.

Effects attributable to long-term exposure are identified by contrasting disease prevalence in high and low exposure communities. An acute effect, such as excess acute respiratory disease, may be a manifestation of chronically impaired resistance to disease. Persistence of illness excess or of altered physiology in a high exposure community provides a means to discriminate between effects attributable to short-term and to long-term exposure. Disentangling the effects of dose-rate, that is, large dose in short intervals vs. repeated small doses over long periods, is difficult in community studies and generally requires controlled experimentation.

Many factors contribute to community differences in the distribution of diseases associated with air pollution exposure. These factors which, along with environmental pollution, co-determine the quantitative level of a health indicator in a community are called "covariates." Important covariate information obtained in the CHESS program includes age, sex, race, education of parents, family size, occupational dust and fume exposure, cigarette smoking habits, geographical migration, and previous illness experience. Either the covariates are kept constant across study groups by the selection of participants, or they are measured; and then, by proper statistical application, it is possible either to isolate or compensate for them.

Environmental Monitoring. Air monitoring stations are sited in each CHESS community to provide credible estimates of pollutant exposure for the study population. The large majority of study subjects live within 1.5 miles of the monitoring station.

Manual instruments operated seven days each week monitored 24-hour integraged concentrations of pollutants. Beginning last January, automated methods listed in Table 3 were introduced into some CHESS stations, and the entire monitoring program will be automated in 1973. Automated stations will telemeter real time pollutant measurements to a central processing station, providing data to relate short-term environmental variations to health indicators of acute response.

CHESS Study Strategies. CHESS area sets are selected to evaluate existing air quality standards for particulates, sulfur oxides, nitrogen oxides, and photochemical oxidants. Because the effects of short-term CO exposures are more precisely studied in controlled exposure chambers, a CHESS area set to measure CO effects was not established.

Middle class neighborhoods are chosen because they represent a large proportion of the population and they have a more homogenous family and social class distribution, and these characteristics change more slowly than in central city neighborhoods.

Family participants are recruited from elementary and secondary school enrollments in CHESS neighborhoods. Recent school busing has complicated recruitment of families living in one neighborhood. Subjects for asthma, cardiac and chronic respiratory disease panels are obtained from responses to the chronic respiratory disease questionnaire and from patient listings of private physicians. Depending on the health indicator, families and panel members are contacted through single-time prevalence surveys, mailed weekly diaries, bi-weekly telephone calls, or telephone calls during episodes. Lung function of school children is measured at each participating school; volumetric methods are employed for these surveys. Hair samples obtained from family members are routinely analyzed for concentrations of zinc, cadmium, lead and copper, and selectively for other trace metals. Maternal-fetal tissue sets, including cord blood, maternal blood, maternal hair and placental tissue, and autopsy tissues are also obtained from each CHESS neighborhood for pollutant burden studies. Cooperation of local hospitals is solicited for tissue collections.

It is expected that the CHESS programs will operate for three to five years in most areas. Measurement of sensitive health indicators during an interval of improving air quality is an optimal way to quantitate the health benefits of pollutant controls. Studies may be extended to detect time lags between air quality improvement and anticipated health benefit. Development work is now in progress to deploy more sensitive and objective health indicators, particularly physiologic and biochemical responses, of short-term variations in air quality.

Results

The CHESS program has been in operation for a little more than two years, and recent summaries of data have given the following results:

I. Short-term exposure

A. 365 ug/m^3 SO$_2$ or more

1. Major episodes at high levels of exposure have demonstrated that air pollution can cause increased numbers of deaths. However, dose-response data are not obtained from these episodes and none were observed during the previous two years in our CHESS areas.

B. Less than 365 ug/m^3 SO$_2$

1. In New York City, symptom ratios for cough, chest discomfort, and restricted activity increased during short episodes of high SO$_2$ pollution over those occurring on low pollution days as follows:

	SO$_2$	TSP		Symptom Ratio		
				Cough	Chest Discomfort	Restricted Activity
Low Pollution	104(.04)	52	Father	1.0	1.0	1.0
			Mother	1.0	1.0	1.0
			Children	1.0	1.0	1.0
High Pollution Episode of <3 Days	340(.13)	192	Father	2.0	6.0	11.0
			Mother	1.8	7.0	10.7
			Children	1.4	6.0	3.5

Non-smokers consistently reported fewer symptoms than smokers but were equally affected by the episode.

2. In New Cumberland, West Virginia, ashtma attacks in a group of asthmatics increased as pollution increased:

	SO$_2$	Asthma Attack Ratio
Low Exposure Day	182 (.069) or lower	1.00 (.364)*
High Exposure Day	183 (.07) or above	1.23

* Daily attack rate per person per day.

Temperature variations had the strongest effect on daily attack rate. After temperature effects were removed, residual daily attack rates were significantly correlated; this relationship was strongest with suspended sulfates. Air pollution effects were strongest at moderate temperatures.

3. Increases in asthma attacks in New York City (143 subjects):

	SO_2	Sulfates ($\mu g/m^3$)	Attack Ratio
Low Exposure Day	≤60 (.023)	---	1.00 (.213)
Intermediate	61-80 (.024-.029)	---	1.07
High Exposure Day	>80 (.03)	---	0.97
Low Exposure Day	---	≤8.0	1.00 (.198)
Intermediate	---	8.1-12	1.04
High Exposure Day	---	>12	1.07

4. In Salt Lake City, asthma attacks increased as pollution increased. This study included 243 children and adults:

	SO_2	Sulfates ($\mu g/m^3$)	Attack Ratio
Low Exposure Day	≤60 (.023)	---	1.00 (1.48)
Intermediate	61-80 (.024-.029)	---	0.96
High Exposure Day	>80 (.03)	---	1.12
Low Exposure Day	---	≤8.0	1.00 (1.39)
Intermediate	---	8.1-12	1.10
High Exposure Day	---	>12	1.16

Temperature was a highly significant covariate. Once effects of temperature were removed, rates could be correlated with suspended sulfates or TSP, more strongly with SO_2.

Without high sulfates, attack rates began to increase when TSP was about 75 $\mu g/m^3$, but when suspended sulfates were high, attack rates began to increase when TSP exceeded the annual secondary standard (60 $\mu g/m^3$).

5. In New York City, increased irritation of symptoms occurred in elderly panels with heart and lung disease (800 subjects over 60 years old):

	24 hr SO_2	Sulfate	Symptom Ratios SO_2 Suburban	Symptom Ratios SO_2 Urban	Symptom Ratios Sulfate Suburban	Symptom Ratios Sulfate Urban
Well	<60	<8	1.0 (7.1)	1.0 (16.5)	1.0 (7.3)	1.0 (14.3)
	60-80	8-12	1.04	0.96	1.0	1.22
	>80	>12	1.39	1.12	1.05	1.25
Heart and Lung Disease	<60	<8	1.0 (19.6)	1.0 (51.9)	1.0 (17.8)	1.0 (48.7)
	60-80	8-12	1.46	0.92	1.18	1.06
	>80	>12	1.23	1.0	1.40	1.09
Estimates for Total Elderly Population	<60	<8	1.0 (7.4)	1.0 (23.0)	1.0 (7.5)	1.0 (20.8)
	60-80	8-12	1.01	0.98	1.01	1.13
	>80	>12	1.25	1.06	1.08	1.18

The most pronounced and consistent effects were associated with temperature. Urban subjects appear to have had greater initial illness.

6. These results confirm the fact that sensitive segments of our population are sensitive to relatively small daily fluctuations in air pollution levels.

II. Long-term exposure

A. Mortality and morbidity - exposure ≥120 $\mu g/m^3$ annual mean

1. In New York City, non-smoking adults in high exposure areas had chronic bronchitis rates 2.4 to 3.9 times those for non-smokers in low exposure areas. For smokers, the relative excess was 1.3 to 1.6 in high exposure areas:

	Annual (68-70) SO_2	Annual (68-70) TSP	Chronic Bronchitis Prevalence Non-smokers Female	Chronic Bronchitis Prevalence Non-smokers Male	Chronic Bronchitis Prevalence Smokers Female	Chronic Bronchitis Prevalence Smokers Male
Suburban	28	35	1.0 (2.1)	1.0 (4.7)	1.0 (10.2)	1.0 (14.0)
Urban I	175	80	3.62	3.89	1.62	1.48
Urban II	230	110	2.38	3.91	1.34	1.51

2. In Chicago, a survey of 50,000 military recruits shows that those living in the Chicago/NW Indiana conurbation had chronic bronchitis rates 10 to 21 percent higher than those coming from outstate non-urban areas. Significantly higher rates were found in both blacks and whites.

   Pollution levels in the urban area were little above national annual standards during the current year, but past exposure had been significantly greater.

3. In Salt Lake City, chronic bronchitis prevalence among 7500 parents of elementary school children were 1.7 to 2.0 times higher for non-smokers and about 1.4 times higher for smokers when living in an area of higher SO_2 exposure; annual mean 108 $\mu g/m^3$ (.04 ppm) and low TSP (68 $\mu g/m^3$).

	Annual Mean		Chronic Bronchitis Prevalence			
			Non-smokers		Smokers	
	SO_2	TSP	Female	Male	Female	Male
Clean Areas	36	50-80	1.0 (3.2)	1.0 (3.3)	1.0 (19.5)	1.0 (15.9)
Polluted Area	108	68	2.03	1.7	1.4	1.39

   Occupationally exposed individuals were excluded from the analysis.

4. In Montana and Idaho communities, increased chronic bronchitis prevalence rates were observed among 4200 parents of elementary school children when annual mean SO_2 exposure exceeded 236 $\mu g/m^3$ (.09 ppm).

	Annual Mean		Chronic Bronchitis Prevalence			
			Non-smokers		Smokers	
	SO_2	TSP	Female	Male	Female	Male
Clean Communities	10-71	50-119	1.0 (1.2)	1.0 (1.0)	1.0 (17.0)	1.0 (11.3)
Pollution Communities	236-275	48-82	2.92	2.40	1.09	1.16

5. A three-year study in Montana and Idaho demonstrated increased frequency of acute LRD and croup in children aged 1 to 12 compared to a control group. After rates were adjusted for age, sex, and socioeconomic factors, significant increases in the frequency of repeated LRD and croup were found in children who had lived in the pollution area for three years, but not in newly arrived children.

	Annual Mean		Symptom Ratios		
	SO_2	TSP	Pneumonia	Croup	Bronchitis
Low Exposure	10-80	50-112	1.0 (5.2)	1.0 (6.2)	1.0 (14.9)
High Exposure	236-275	54-102	1.1	1.01	1.06

B. Morbidity - annual exposure of 80 to 120 µg/m³

1. In Salt Lake City, excess acute LRD frequency was observed in children 0 to 12 years old who had been residents for three years or more in an area of high pollution, but not in children with less than three years of residence.

	SO_2 µg/m³	TSP µg/m³	LRD	Pneumonia	Croup	Bronchitis
Low Exposure	5	77		One episode or more		
			1.0 (27.3)	1.0 (4.4)	1.0 (16.9)	1.0 (16.5)
				Two or more episodes		
			1.0 (15.2)	1.0 (1.2)	1.0 (8.0)	1.0 (6.5)
High Exposure	108	68		One episode or more		
			1.4	1.09	1.56	1.53
				Two or more episodes		
			1.54	1.0	1.73	1.66

2. In Chicago, members of volunteer families experienced a 30 to 50 percent excess frequency of acute LRD in the higher SO_2 area. (106 µgSO_2/m³ = high exposure, 57 µg/m³ = low exposure.)

C. Morbidity - exposures of less than 80 µg/m³

1. In New York City, members of families experienced a 20 to 40 percent excess frequency of acute LRD in the higher exposure areas. Additionally, these families experienced increased severity of their LRD.

	Annual		Relative Risk of Acute LRD			
	SO_2	TSP	Pre-school Children	School Children	Mothers	Fathers
Low Exposure	23	34	1.0 (4.59)	1.0 (3.51)	1.0 (2.28)	1.0 (1.80)
High Exposure	50-88	87-104	1.42	1.21	1.23	1.24

Parental smoking was associated with increased frequency of LRD and severity of LRD in children. Gas used for cooking or in space heating was associated with increased severity but not increased frequency in mothers and children.

2. In New York City, ventilatory function was decreased in children 9 to 13 years old who had experienced high pollution during the period of their rapid lung development. Function ranged 2.4 percent below that of children from less polluted areas.

3. In Cincinnati, ventilatory function was decreased in children 7 to 8 years old living in the high pollution exposure areas. In this study, function was decreased about 15 percent and was associated with suspended sulfate concentrations.

Conclusion

The significance of these studies is obvious. They confirm the fact that higher rates of acute and chronic illnesses are associated with moderate increases in pollution. It is not possible at this time to attribute all of this excess illness to present levels of pollution since the effects of prior exposure are unknown. However, the current studies amply support the levels established as ambient air quality standards.

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TERATOGENESIS AND MUTAGENESIS OF ENVIRONMENTAL CHEMICALS

Hans L. Falk

If a discussion of the carcinogenic potential and the threats to man's health by environmental chemicals is full of ambiguities, the situation regarding mutagenic and teratogenic hazards is even more complicated.

I should like to start with separation of mutagenesis and teratogenesis, two hazards often mentioned in one breath but which involve different mechanisms and usually relate to different types of chemicals. They are always bunched together under the heading of human birth defects because it may be impossible to identify the mechanism involved without further study.

Environmental Chemicals as Teratogens

The problem of teratogenesis has been brought to everyone's attention following the publication of the studies on 2,4,5-T and its follow-up, which I shall mention to point out some of the difficulties which have been encountered. This compound was tested as one of a group of pesticides without any anticipation of health hazards or suspicion. The commercially available product was chosen for the study. Because of extensive use of the herbicide--in 1964 in the U.S., 1.5 million pounds were used on farms and 7.3 million pounds were for non-farm use, mostly for right-of-ways, a total of nearly 9 million pounds--it was felt to be of importance to study its potential toxicity.

At the time the studies were initiated, the use of 2,4,5-T in formulations for military defoliation in Vietnam was not general knowledge and it was used to a very minor extent. The herbicide was used in the U.S., as permitted by USDA regulation, on apples, blueberries, cereal grains, sugar cane, rangeland, lakes and ponds.

One difficulty in its identification was the non-specific description of the products as 2,4,5-T when it was used as the acid, as a salt, or as a variety of esters. It was realized that this could cause complications, but not the complication actually encountered. As it was assumed that the acid would be less toxic than some of the esters, it was used in that study. It was tested in two strains of mice at three dose levels and administered, orally or by subcutaneous injection, during days 6-14 or 9-17 in C57 black mice, or on days 6-15 in AKR mice. The results showed statistically significant increases in cleft palates and cystic kidneys in the fetuses.

Because of the positive results in mice, tests were also carried out on Sprague-Dawley rats given orally at three dose levels from 4.6 to 46.4 mg/kg from day 10-15 resulting in cystic kidneys in these fetuses. The highest dose administered produced fetal mortality so that inadequate information on malformations was obtained in that group. Very striking was the observation of hemorrhages in the gastro-intestinal tract of rat fetuses which showed a dose-response relationship. It was of interest that 2,4-D did not produce similar teratologic effects; only some of the esters showed weakly positive results.

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At this stage, industry helped to clarify the situation by analysing the sample of 2,4,5-T that had been used for these studies and detecting contamination of the sample with a highly toxic compound, 2,3,7,8-tetrachlorodibenzo-p-dioxin. This compound had in the past been held responsible for the production of chloracne in a number of workers involved in the synthesis of 2,4,5-T, particularly in the cleaning operations of apparatus. Following this discovery, the process of 2,4,5-T synthesis was altered slightly so as to avoid the production of the dioxins. This discovery was made and remedial action taken after the samples for these studies had been obtained, and the investigators were not aware of the contaminant or its partly known toxicity.

With this information and the collaboration by industry, the impurity was obtained for study, as was technical 2,4,5-T of recent vintage and a particularly pure sample of the herbicide without detectable dioxin contamination. Other impurities present in commercial 2,4,5-T were also identified.

The results of these follow-up studies were very interesting. The increased incidence of defects and fetal mortality in the rat could be blamed entirely on the impurity (the dioxin). The hemorrhagic GI tract in mice was also caused by the impurity. However, even the highly purified 2,4,5-T still produced cleft palates and renal defects in mice. The dose required, however, was very high: 100 mg/kg subcutaneously in DMSO as solvent given to Charles River mice from day 6-15 of pregnancy. In contrast, kidney involvement was seen with dioxin at the 1 µg/kg dose level in the same experimental setup.

These studies seem to have come to a successful conclusion; namely, the identification of a very toxic impurity which has, for other reasons, already been eliminated from the present-day 2,4,5-T herbicide, leaving the product, if not completely clean, at least of negligible toxicity. However, a survey made recently of a number of pesticides by USDA scientists revealed that several pesticides derived from chlorinated phenols contained sizeable amounts of various chlorinated dibenzo-p-dioxins. Thus, samples of pentachlorophenol had high levels of octa- and hepta-chlorodibenzo-p-dioxins with lower amounts of hexachloro and fewer chlorinated dioxins in 10 out of 11 samples. The dioxin levels were very high, between 10 and 1000 ppm of the higher chlorinated dioxins. The samples of tetra- and trichlorophenol tested were far lower in dioxins.

In recent times, the contamination of PCB's with chlorinated dibenzofurans and dioxins in Europe and the U.S. has been documented so that the problem has not disappeared as yet. However, much greater emphasis has been placed on the hazards of this type of contamination, so that it will be looked for and eliminated. The possibility that new antibacterials may contain or give rise to dioxins must be taken into consideration when hexachlorophene will have to be replaced by other antibacterials.

Although enough information appears to be available on the rates of degradation of 2,4,5-T herbicides, little information exists for dioxin degradation, except for one report on photodecomposition of chlorinated dibenzodioxins. In this study, decomposition on exposure to ultraviolet light or sunlight was observed *in vitro* on solution of the dioxin in alcohol, but no such luck was had when the material was present in aqueous suspension or in soil.

The hazard that may arise when chlorinated phenols or herbicides like 2,4,5-T are exposed to conditions that may cause their transformation to dioxins must be carefully evaluated. Such a hazard may not depend on the optimal pH for dioxin formation but could be present on pyrolysis of large quantities of the herbicide even though at a very low yield.

The considerable attention paid to 2,4,5-T as a potential teratogen has resulted in ignorance of other environmental chemicals that may have similar toxicologic properties. In the large scale screening which detected the herbicide 2,4,5-T as teratogenic, some others were also found to be active, particularly pentachloronitrobenzene (PCNB). PCNB produced renal agenesis in C57 black mice when administered from day 6-10 of pregnancy at 460 mg/kg; when administered from day 10-14, this effect was not observed.

Sevin, Captan, and Folpet were also found to be teratogenic to some extent, but less strikingly so, even though the appearance of abnormalities was statistically significant. Many other insecticides, among them the chlorinated hydrocarbons and the organophosphates, were also teratogenic in a number of test species. So were mercury compounds, including methylmercury.

Toxic chemicals which the mammalian organism has little capacity to excrete or detoxify in its organs and tissues may turn out to be teratogenic. Under conditions where detoxification and elimination are prevented, the transfer from the mother to the developing fetus is one of the only routes of elimination of the chemical. Experimental data on teratogenesis exists for such chemicals, i.e., mirex, the chlorinated dibenzo-p-dioxins, the polychlorinated biphenyls (PCB's), and methylmercury. It will be true for other environmental chemicals which are lipid soluble and hard to detoxify and conjugate.

When reviewing the literature, including the Report of the Secretary's Commission on Pesticides and Their Relationship to Environmental Health, one becomes concerned about the potential teratogenicity of many environmental chemicals. This is particularly true when the chick embryo is chosen as the test animal. Criticisms have been raised against the use of that species because the introduction of any foreign chemical is final. Unlike the mammalian fetus, the chick embryo cannot eliminate the chemical successfully. The choice of a mammalian species circumvents that situation and makes the experiments more extrapolatable. After an early "successful" attempt to duplicate the teratogenicity of thalidomide in the chick embryo, one investigator replaced the insoluble drug by an equivalent number of grains of sand and produced the same teratogenic effects. However, even in the case of soluble chemicals, the chick can only serve as a simple screening test for chemicals without immediate follow-up by legislative action.

Considerable efforts are being made to come up with adequate test systems. The results obtained with 2,4,5-T serve to illuminate some problems quite clearly. Besides the purity of the compound, the choice of species and strain is important. The data on the rat, which are negative, can be contrasted with those on the mouse, which are positive; further studies may determine if mouse or rat is closer to man.

Sometimes the ability to eliminate the compound through the kidney is the crucial event; in other situations, it is the binding to plasma proteins that may make the difference in toxicity. Thus, it is important to determine some of the pharmacologic parameters for experimental animal species and for man before carrying out too many experiments and blindly using too many species or strains. However, it must be recognized that, within a species, the physiologic, pharmacologic, or biochemical characteristics of the adult may differ significantly from those of the fetus.

One of the problems deals with the timing of the chemical insults. The mouse and rat have a very short organogenesis time which does not allow for very careful pinpointing of the time of the actual interaction. These species are, therefore, very useful for quick answers on potential teratogenic hazards. However, they are less suitable to answer questions on specific times during pregnancy which may be more or less sensitive to chemical insults. Therefore, there is a need for additional studies, once a chemical has been found to be teratogenic to mice or rats, with species with longer pregnancies to determine the sensitive period for better extrapolation to man.

Evidence of human teratogenicity due to environmental chemicals is quite sparse and particularly inconclusive regarding the effects of chlorinated hydrocarbon insecticides or organophosphates. High dose exposure to PCB's and methylmercury, on the other hand, shows the teratogenic effect of these chemicals taken with the food. From such data, it was possible to determine intake levels that were without effect on the developing offspring.

One can conclude that teratogenic effects in humans have not been observed unless the intake of the environmental chemical was exceedingly high. Among these environmental chemicals, one encounters some that resist degradation in the environment or in the mammalian organisms and that accumulate in tissue and will reach the developing fetus transplacentally or the young infant through his mother's milk. A few toxic chemicals fall into this classification, and introduction of these chemicals into man's environment must be avoided by all means.

Environmental Chemicals as Mutagens

Mutagenic chemicals in man's environment may represent the most serious health hazard. They deserve attention of the highest priority before the ill-effects may become apparent in future generations when no remedial steps may be available to turn the clock back. It is not at all certain that we have already encountered such a situation of exposure to mutagenic chemicals, but this situation may arise at any time, if it has not happened yet.

A consideration of mutagenic hazards for man must take into account all factors possibly involved in producing this hazard for man and cannot rely on evidence only that the chemical possesses mutagenic properties for some microbial test system. The test systems that have been used in the past and that are still in use today are very sophisticated, but they are limited in application when extrapolation to man is required. Systems that determine effects on bacteria, fungi, viruses or mammalian cells in tissue culture suffer from shortcomings relating to the lack of potential barriers between the chemical and the genetic material, which is quite complicated in the mammalian species. Conditions also differ with regard to the defense systems available in the mammalian organism which are at work and may completely alter the hazard. On the other hand, certain chemicals which become activated to mutagenic compounds by mammalian metabolic steps would not give evidence for mutagenicity in the above-mentioned systems.

The simple systems mentioned above nonetheless serve their purpose; namely, to differentiate between mutagenic chemicals that are effective without metabolism and those that need activation, which are not detected in these simple tests. Their main purpose, of course, is to have a first screen. If the result is negative, it does not allow immediate approval of the chemical for large scale use; and if positive, it similarly does not suggest legal action as the next step without any further testing.

Looking at pesticides, for example, it is apparent that use is made of some chemicals on the basis of their mutagenic properties. We would, therefore, have to conclude on the basis of microbial tests that these chemicals must be removed. Chemosterilants, which are used together with ultraviolet light, insect pheromones or other attractants for insect control, will kill, sterilize or cause genetic damage. The compounds have been studied exhaustively and qualify as potent mutagens. The question, therefore, is whether, under the conditions of usage, a hazard may exist for man and other species. The chemosterilants are highly toxic chemicals also for man. However, they tend to break down and the rate of degradation has been determined which showed that, although the parent compound was readily degraded, alkylating activity was still present in the insect after some time. The activity was due to ethylene imine, a breakdown product of the chemosterilant. As the insect may serve as food for other animals, it is possible that some hazard may be transmitted to the predator, be it a fish or a bird; but it is not likely to reach man through the food chain under most conditions.

Other pesticides, particularly the herbicides, may exert their function by interfering with cell replication. Results show the appearance of chromosomal breaks in many plants when exposed to herbicides. The herbicide may at first still be in its active form in the plant, although its metabolism in the plant tissue will lead to its inactivation. When it remains intact, as in the case of maleic hydrazide, it could present a hazard to man in some food staples. However, the amount of the compound used and the residues encountered in food staples are rather insignificant.

Fungicides also proved mutagenic, and the list of potential mutagenic chemicals among the pesticides is large. This high probability cannot be applied across the board to other groups of environmental chemicals, because the chemicals selected for pesticidal use were, of course, lethal to the organisms to be controlled, frequently affecting their reproductive capabilities.

For each of the pesticides, additional studies are needed to determine whether the mutagenic effect will reach man on environmental exposure to the chemical, as contrasted with occupational exposure.

Fortunately, many investigators have asked relevant questions and found answers, so that we can evaluate the probability of these chemicals reaching man intact to present a mutagenic hazard. Pesticides as the fumigants are very reactive and will not be mutagenic after they have reacted. Acrylonitrile, hydrogen cyanide, formaldehyde, ethylene oxide, betapropiolactone, methyl bromide, and methylene bromide belong to this group. The reactivity of these chemicals does not, however, imply that there is no health hazard; although the mutagenic property is lost, the reaction products may be toxic.

The same situation applies to fungicides such as Captan or the metallodithiocarbamates which readily decompose and thus represent less of a mutagenic hazard. Other fungicides, such as methylmercury, however, maintain their toxicity. In general, every pesticide has to be studied separately to determine the reactivity of the compound in its ecologic system and the rate of degradation in mammals if it reaches them in the food chain.

Once the chemical has been ingested, it is not certain that it will reach testes or ovaries; and it has been documented that the chemical does not cross the barrier that easily; so an extrapolation from the microbiological or mammalian *in vitro* system is not justifiable.

However, among the chemicals that represent a potential hazard, the most problematic ones are those that are not very reactive and may have a considerable biologic half-life, and those that are precursors of mutagenic compounds and require metabolism before being activated. These compounds may play havoc in mammals, yet would not be detected in the general test systems mentioned before. Adequate test systems required for their detection are being developed and will soon be available.

In order to respond to this need, the *host-mediated assay* was developed which introduced into mice the microorganism and the potential mutagen which may require activation by the mammalian organism.

A few problems remained with the host-mediated assay, however. First was the problem that the host did not take kindly to the invasion by microorganisms and by mobilization of macrophages would soon annihilate the microorganisms. The time for the experiments was, therefore, limited; and the conditions for survival of the cells were not good. A second problem was the need for metabolites to become available to the microorganisms; and the possibility exists that metabolism was too slow, that metabolites would be further modified, i.e., conjugated or eliminated by the kidney, giving the impression that no mutagenic compounds were formed. Tests would be negative also if the compound was excreted in the bile to be reabsorbed into the circulation after hydrolysis, bypassing the peritoneal cavity and its occupants. Its mutagenicity would be missed.

The newly developed host-mediated assay utilizing mouse cells introduced into the murine peritoneal cavity in a semipermeable chamber will take care of some of the problems; however, the body's defenses may close off the membrane, so that after awhile the metabolized chemical, if present in the peritoneal cavity, may not reach the cells.

When considering the number of chemicals that possess mutagenic properties, we must realize that the organism possesses defense mechanisms that are fast at work to repair the damage to DNA. It is not the exception but the rule that damage to DNA by crosslinking or single hits is rapidly detected and repaired by enzymes which have several mechanisms at their disposal to excise the damaged parts and to repair them. The repair of ultraviolet-damaged DNA (formation of thymine dimers) has been examined and the enzymes involved have been characterized. In organisms in which these repair enzymes are absent, damage may be permanent and lethality and mutagenesis are much enhanced. Similarly, in humans suffering from xeroderma pigmentosum, the repair enzymes are deficient and cells from the skin of these patients demonstrate lack of excision of dimers and lack of appearance of short chains of DNA, which are normally formed during repair and disappear when normal cell repair is completed.

If we are reassured that for most humans this risk of missing repair mechanisms does not exist and that other repair mechanisms actually exist in addition, it comes as a shock that synergism has been observed, i.e., a greater mutagenic risk when certain chemicals are introduced which will interfere with repair mechanisms. So far, only a few chemicals have been found to interfere with the repair mechanisms, but only very few have so far been tested.

It seems clear that a search for mutagenic chemicals in the environment must be initiated so that we will know which chemicals might present potential risks. However, after this primary screen, we must determine the probability that the chemical could reach the target tissue in a high enough dose to be an effective mutagen. It is not true that one molecule would be sufficient to cause a mutation considering side reactions with other molecules, metabolism and detoxification of the mutagen, and the varieties of cells that may be hit by the mutagen. Even if the "proper cell" in the "proper stage" has been affected and the result is a point mutation, it is not at all certain that the DNA strand which has been hit is part of an active gene, or that the base is in a crucial position where change will alter the translation of the code, or that the replacement of the amino acid by a different one will produce changes that are functionally important, or that the mutation will not be repaired.

On the other hand, if the mutation is carried along, and even if it is only of minor importance, it may persist without producing any lethality; and many may accumulate as recessive mutations until the load of these multiple minor modifications leads to true deficiencies and difficulties in future generations.

We need to perfect test systems, to initiate screens, and to initiate all the other biochemical and physiologic studies to determine the biologic half-life and the chances of receiving a load of undesirable genetic traits; and we must take action to eliminate the hazardous chemicals from common use.

PESTICIDES IN AIR

Lawrence H. Mounce

Pesticides are one of several classes of toxicants which are present in our atmosphere. This class of toxicants is in contrast with some of the domestic and industrial contaminants which merely add to compounds naturally occurring in air. Pesticides, which are generally regarded as those synthetic chemicals having been produced since World War II and used as insecticides, herbicides, and fungicides, are original air pollution burdens which can alter the quality of air.

Air is one of the three main reservoirs for pesticide residues with the other two being soil and water. Much investigational and monitoring work on pesticides in soil and water as well as vegetation, wildlife, and humans has already been conducted; however, much less knowledge has been accumulated on the incidence and levels of pesticides in air. This is in part due to the small sample sizes available because of the great volume and dilution factor of air, and partly due to the lack of adequate sampling and analytical methods. Despite these limitations, there is an increasing body of data to indicate the universal presence of pesticides in air. The significance of these airborne pesticides to man, the environment, and the ecosystem is less clear.

Three important reports on the subject of pesticides were prepared by national committees or commissions and released in the year of 1969. Significant statements relating to pesticides in air from these reports are as follows:

1. The report of the Committee on Persistent Pesticides of the National Research Council dated May 1969 states the following: "Although the technology of air sampling is not well advanced, air sampling must be included in any effort whose aim is to monitor the health-related aspects of man's immediate environment. Further, the absence of quantitated information about transport of pesticides in the atmosphere is a critical deficiency in our understanding of the biosphere circulation of persistent materials."

2. The report "Cleaning Our Environment: The Chemical Basis for Action: by the American Chemical Society lists the following recommendations:

   "P-1: Pesticide Monitoring Programs on all phases of the environment should be continued. In the case of air...the present programs should be extended."

   "P-2: For purposes of chemical analysis, research should be pursued on the development of more adequate methods for the separation of minute amounts of pesticides from water and air."

   "P-3: Research should be expanded on the toxicity of pesticides when they are inhaled as opposed to dermal exposure or oral intake."

3. The report of the Secretary's Commission on Pesticides presented 14 broad recommendations. One of these reads as follows: "Develop suitable standards for pesticide content in food, water and air..."

I believe these conclusions and recommendations point out the unknowns, problems, and challenges with respect to pesticides in air.

Not only are pesticides originally-occurring foreign substances in air, but they are also intentionally injected through the air to crops and other target objects, whereas most other airborne contaminants are incidental to transportation or the production of power.

Pesticides may enter the air in several ways. These are:

1. Drift and volatilization from aerial or ground applications.
2. Volatilization from treated soil, plants, or water.
3. Wind translocation of particulate matter such as soil and plant fibers.
4. Domestic use of aerosols and thermal vapors incidental to the production and formulation of pesticides.

Drift. One of the first and probably the most familiar problem relating to airborne pesticides is the occurrence of drift from the target crop to non-target areas. The application that obliterated the cucumber patch as well as the weeds in the adjacent wheat field, and the withering flower bed adjacent to the neighbor's weed-free lawn are familiar stories. Such economic and aesthetic effects are immediately apparent. On the other hand, drift onto non-target food crops may not be visually apparent but has been an avenue of chemicals entering the food chain and had resulted in the seizure and condemnation of human and animal food crops. After World War II, crop dusting, both aerially and with ground rigs, was a common practice; but now the term "cropduster" is becoming a misnomer as it is becoming hard to find an applicating firm that will apply a chemical in dust form. This is indicative of the greater drift problems associated with dust application and the greatly increased ability of chemists. Crop dusting has given way to spraying. Refinements in the design of agricultural aircraft, better control of the spray through calibration of particle size, and improved formulations of chemicals have all contributed to reducing the problems associated with drift. Newer forms of application, such as the use of granules and foam, show promise for further reducing drift and the contribution of pesticides into the air.

Many studies have been made concerning the factors involved in drift. One of these studies has shown the difference in drift between a spray and a dust application. DDT was applied to a field and its concentration was then measured one-half mile downwind. The dust concentration was 1.4 ppm, whereas the concentration from the spray was only 0.1 ppm. This and other studies have shown that drift is related to particle size, with the sprays being in the 150-200 μ range and dust being in the 10-20 μ range.

Volatilization. Volatilization of pesticides from treated soils, plants, and water is dependent on complex interrelationships involving properties of the chemical, the soil, and the climate. Chemical properties which are important include acqueous solubility, vapor pressure, and the intrinsic stability of the compound. The absolute vapor pressures of the organochlorine insecticides are very low, yet volatilization is one of the most important causes of loss from the soil, and this is enhanced in the presence of free water at the soil surface. Vaporization is a continuous process throughout most of the year; and the rate of release will depend upon such factors as temperature, soil tillage, type of crop cover, and similar factors.

Translocation. In 1966, Cohen and Pinkerton (2) reported the transport of pesticide-laden dust through the atmosphere. This dust originated in a mammoth dust storm over the Southern High Plains west of Lubbock, Texas, and was subsequently deposited by rainfall many hundreds of miles away 24 hours later. Results of analysis are given in Table 1.

TABLE 1 (2)

Pesticide Content of Dust

Pesticide	Concentration (ppm)[1]
DDT (tech.)	0.6
Chlordan (tech.)	0.5
DDE	0.2
Ronnel	0.2
Heptachlor epoxide	0.04
2,4,5-T	0.04
Dieldrin	0.003
Total organic chlorine	1.34
Total organic sulfur	0.5

[1] Based on air-dried weight of dust.

Pesticides or related products may be discharged directly into the air through smokes and other clouds incidental to manufacturing or processing or in burning of "empty" containers, or even in the case of fires in warehouses and such situations. Wolfe *et al.* (10) have reported finding as much as 7.9 mg/m^3 of parathion in such smoke.

The distribution and dispersion of airborne pesticides will depend on particle size and meteorological conditions as well as distance above ground at which they become airborne. Information on the dispersal and distribution of pesticides in the vapor phase or in the particle range below 5 μ is almost completely lacking.

The Division of Pesticide Community Studies of the Environmental Protection Agency has been conducting a monitoring program for the past two years to determine the incidence and levels of pesticides in the air on a national basis. This is known as the Air Monitoring Program and is under the direction of Dr. Anne Yobs. This program has collected 1,050 samples over a two-year period from 45 sites throughout the contiguous 48 states with one sampling site in the Bahamas. I consider this to be the most comprehensive air sampling program for pesticides.

One of these sites is located at the Colorado Project in Greeley. The type of sampler used is one which was developed by the Midwest Research Institute and is of the impinger type whereby air is bubbled through ethylene glycol trapping both particulates and gases. The results show that DDT and one of its metabolites, Dieldrin, and two isomers of BHC were found in more than half the samples. Two isomers of DDT and Dieldrin were found at all sites last year. You may note that all these compounds mentioned are in the more persistent class of chlorinated hydrocarbons. Other pesticide chemicals have been detected in the chlorinated hydrocarbon group as well as organophosphate pesticides, but with less frequency. These results are found in Table 3 (13).

The national levels do not approach the American Congress of Governmental Industrial Hygienists' threshold limits. A wider variety, although not the highest levels, of pesticides have been found in the urban and commercial areas as compared to residential, agricultural, or rural areas (Table 2).

TABLE 2 (13)

Average Number of Pesticides Reported by Land Use

	1970	1971
Urban commercial	13.2	12.3
Rural farm	11.0	12.0
Urban residential	10.8	11.0
Urban agricultural	10.0	10.2
Rural non-farm		8.2

The Colorado Project, in addition to the air monitoring work, has pursued the subject of pesticides in air from a slightly different approach. Prior work at our project with three groups of families (an urban control, rural and pesticide-exposed worker family groups) involved the analysis of environmental samples such as soil, water, housedust, and diet as well as blood samples from these groups. Housedust was the only variable showing any association to blood residues of the family members from two sets of such data. This suggested an environmental influence and the possibility of residue deposition in housedust from the air seemed strong.

TABLE 3 (13)

Incidence of Pesticides in Ambient Air, 1971

Pesticide	No. Times Reported	Percent Samples Positive	No. Sites Reporting
p,p'-DDT	936	96.7	45
p,p'-DDE	900	93.0	43
Dieldrin	842	87.0	44
o,p'-DDT	769	79.4	42
α-BHC	678	70.0	27
δ-BHC	563	58.2	24
Heptachlor	265	27.4	17
β-BHC	108	11.2	12
Endrin	108	11.2	6
p,p'-DDD	98	10.1	15
Toxaphene	72	7.4	4
Treflan	53	5.5	8
Chlordane & metabolites	47	4.9	10
Dacthal	23	2.4	2
Thiodan	19	2.0	5
Aldrin	17	1.8	3
o,p'-DDE	7	0.7	1
o,p'-DDD	6	0.6	1
Captan	6	0.6	3
Methoxychlor	5	0.5	1
Kepone	3	0.3	1
Diazinon	318	32.9	24
Malathion	136	14.0	19
M. Parathion	54	5.6	8
Phorate	41	4.2	7
Parathion	26	2.7	9
Phosvel	11	1.1	1
Mocap	5	0.5	1
Ronnel	3	0.3	1
Ethion	1	0.1	1
Trithion	1	0.1	1
Folex	1	0.1	1
2,4-D esters	122	12.6	13
2,4,5-T esters	25	2.6	6
Azodrin	4	0.4	1

Total Sites: 45 Total Samples: 968

This was followed by a year-long study involving inside and outside air samples and housedust samples collected monthly from 12 homes of farmers and pesticide workers. More compounds were detected in the inside air samples than in the outside air samples, and the levels were also generally higher. High housedust and inside air residue levels were also associated (Table 4).

Impact

Factors to be considered in the assessment of pesticides in the atmosphere include:

1. With air being the most fluid of the reservoirs, pesticides are more easily and rapidly dispersed.
2. Dispersion into the air reservoir is also the most difficult to control.
3. Pesticides are present, however minute, in the atmosphere and in the air we breathe.
4. The function of respiration is designed for the efficient absorption or exchange of the gases in the air we breathe. It is a continuous and intimate contact. The lungs utilize about 10,000 liters per day; the surface area of the lungs is 80-100 square meters as compared with our body surface of about 1.73 square meters.
5. The potential for reacting with other air pollutants, conversion to other toxic forms, or the production of other compounds must be considered.

TABLE 4 (13)

Percent Incidence of Pesticides in Environmental Samples from Homes of Pesticide Workers

Pesticide	Outside Air	Inside Air	Housedust
Organochlorides			
p,p'-DDT	100	100	99
p,p'-DDE	100	99	22
o,p'-DDT	99	100	48
p,p'-DDD	16	26	8
Methoxychlor	24	11	15
α-BHC	100	44	0
Lindane	79	89	18
Chlordane	95	74	56
Heptachlor	3	4	0
Heptachlor epoxide	11	1	0
Dieldrin	99	99	33
Endrin	75	64	10
Endosulfan	5	19	14
Herbicides			
Dacthal	85	90	48
Organophosphates			
Parathion	55	65	57
Methyl parathion	6	16	27
Diazinon	8	15	30
Ronnel	18	9	26
Ethion	0	14	19

Number of Samples: 149

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
Operations Division, Office of Pesticide Programs
CHAMBLEE, GEORGIA 30341

April 30, 1973

TO ALL COURSE PARTICIPANTS:

The paper entitled "The Effects of Environmental Impact Statements on Federal Pesticide Programs" by William M. Hoffman was omitted inadvertently from the proceedings of the course, "Environmental Chemicals - Human and Animal Health." We regret this omission and enclose a copy of the paper, which may be inserted in your copy of the book following page 200.

Sincerely yours,

Anne R. Yobs, M.D.
Chief, Training & Education Branch

Enclosure

THE EFFECT OF ENVIRONMENTAL IMPACT STATEMENTS ON FEDERAL PESTICIDE PROGRAMS*

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President Richard M. Nixon symbolically chose to sign as his first official act of the decade of the Seventies the National Environmental Policy Act of 1970 (NEPA). At the time it was hailed as "one of the most significant" and "the most important and far-reaching conservation measure ever enacted." Now, some 2-1/2 years later, the Act has established itself as one of the most controversial environmental measures of all times--one whose impact has touched virtually every department of the Federal government and many a Member of Congress in a very short time.

The Purposes of the Act

To declare a national policy which will encourage productive and enjoyable harmony between man and his environment, and to establish a Council on Environmental Quality (CEQ). The Administration originally proposed a cabinet-level coordinating committee but Congress insisted on a statutory council and so CEQ was created as a three-member body appointed by the President and subject to Senate confirmation. By law, CEQ is authorized to:

1. Assist the President in preparing the annual environmental quality report.
2. Gather information on environmental conditions and trends.
3. Review and appraise federal activities affecting the environment.
4. Develop and recommend environmental policy to the President.
5. Conduct ecological and environmental investigations and research, and
6. Conduct such other studies as the President may request.

When the executive order directing implementation of the procedures of the Act was passed, CEQ was given further authorization to:

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*Presented at the course "Environmental Chemicals - Human and Animal Health," sponsored by the Institute of Rural Environmental Health, Colorado State University and Division of Pesticide Community Studies, EPA, August 7-11, 1972, Fort Collins, Colorado.

1. Conduct public hearings and conferences on environmental issues,
2. Coordinate (not merely review and appraise) Federal activities affecting the environment,
3. Issue guidelines to the Federal agencies for compliance with the Act, and
4. Issue such other instructions to the agencies and request such information from them as necessary to fulfill its responsibilities.

The significance of these added authorities is evident. First, they clearly underscore the commitment of the administration as well as the Congress to environmental policy initiatives. Second, they give CEQ powers otherwise held by few if any agencies in the executive office, except Office Management and Budget, to guide and influence the policies and actions of other agencies. Thus, whatever may be said of the actions of CEQ, they have been carried out with a clear mandate from both Congress and the President to harmonize Federal actions with environmental quality goals.

Section 102(2)(C) of the Act requires that any Federal agency proposing legislation or planning to undertake an action "significantly affecting the quality of the human environment" file an impact statement with the Council. The statements describe the legislation or action, its impact, and the alternatives considered. This draft statement must be circulated by the initiating Agency to the public and to appropriate Federal, State, and local environmental agencies. Comments received on the draft statement become a part of the public record along with the final statement, which should reflect the comments.

The draft environmental impact statement must discuss the following topics:

1. A description of the proposed action including information and technical data adequate to permit a careful assessment of environmental impact by the commenting agencies.
2. The probable impact of the proposed action on the environment, including impact on ecological systems such as wildlife, fish, and marine life. Both primary and secondary significant consequences should be included in the analysis.
3. Any probable adverse effects which cannot be avoided.
4. Alternatives to the proposed actions. A rigorous exploration and objective evaluation of alternatives that might avoid some or all of the adverse effects is essential. Sufficient analysis of such alternatives and their costs and impact should be covered in order not to foreclose options which might have less detrimental effects.

5. Relationship between local short-term uses of man's environment and the maintenance and enhancement of long-term productivity.

6. Any irreversible and irretrievable commitments of resources which would be involved in the proposed action should it be implemented.

The Council on Environmental Quality has issued guidelines on how agencies are to meet this requirement (Federal Register 36, 7724 (April 23, 1971)), and most agencies have set up procedures applying the requirement to their own programs. The EPA procedure will be published in the Federal Register within the next few weeks. The Food and Drug Administration published a notice of their proposed impact statement procedures on July 12, 1972.

State governments prepare statements only when their actions are supported by Federal contracts, grants, or permits and the Federal agencies have delegated the preparation authority to the State level. An example is the Federal Highway Administration, which provides matching grants for many State highway construction programs. The draft statements are generally prepared by the State Highway Department; the Department of Transportation takes responsibility for the final statements.

Must industry prepare impact statements? Generally speaking, no. The exception comes when an industry action requires a Federal license or permit--such as a Corps of Engineers dredging permit or a transmission right-of-way across Federal land. When a regulatory permit or action calls for a statement, the Agency will prepare the statement but may require the private industry involved to file a preliminary environmental report analyzing the impact of what it proposes to do.

The draft statements must be made early enough in the Agency review process before an action is taken in order to permit meaningful consideration of the environmental issues involved. In addition, the 90-day waiting period requirement means that the Agency must anticipate a minimum 90-day wait from filing the draft statement to beginning action. To the maximum extent possible no action is to be taken within 90 days after the draft statement has been made available, nor is it to be taken within 30 days of the final statements availability. These waiting periods only apply to actions the Agency can take itself and may be modified with CEQ's consent when emergency circumstances, expense to the government or impaired program effectiveness make modification appropriate.

The CEQ guideline listed earlier contains an appendix which lists Federal agencies with expertise in particular aspects of the environment which should be asked to comment. In the case of pesticides, the following are listed:

Department of Agriculture
    Agricultural Research Service
    Consumer and Marketing Service
    Forest Service

Department of Commerce
    National Marine Fisheries Service
    National Oceanic and Atmospheric Administration

Environmental Protection Agency
    Office of Pesticides Programs

Department of the Interior
    Bureau of Reclamation
    Bureau of Sport Fisheries and Wildlife
    Bureau of Land Management

Department of Health, Education, and Welfare.

In addition, State or local reviews may be relevant. Any individual or organization may also comment on the draft statement: he may express support or opposition, suggest alternatives or point out project effects that may have escaped the attention of its sponsors. These comments may be in the form of a letter, a critique, or even a "counter-statement" setting forth their views and analysis in as great a depth as the original itself.

The Agency preparing the statement is responsible for making it available to all. All draft and final statements are listed in the CEQ publication --102 Monitor, the National Technical Information Service, and the Environmental Law Reporter. Additionally, the CEQ publishes regularly lists of statements in the Federal Register. Copies can usually be acquired from the Regional offices of the Agencies. Many who feel that they want to comment on most statements from a particular Agency have their name on the regular mailing list for commentators.

As the Agency supervising the whole progress, the Council on Environmental Quality must pay special attention to maintaining the rules for the flow of reports, leaving most substantive comments to the particular expert Agency reviewers. The limits of CEQ's powers must also be noted. It has no authority to veto the actions of any other Agency, nor even to require an Agency to file the draft statement. It does have the power to review Agency's actions and the responses of interested groups and to give advice to both the Agency and to the President. But in an intractable conflict with another Agency, CEQ's power is limited to its ability to persuade the President to take action. Its influence can be explained only by its success in persuading the President to adopt its recommendations or by its success in persuading agencies to adopt its recommendations voluntarily and thereby avoid later consequences in the wider forum of congressional, judicial and public opinion.

Federal Agencies have taken, modified, and avoided actions on the basis of the NEPA environmental analysis. For example, the Department of Defense amended plans for munitions disposal. The Forest Service switched from clear cutting to selective cutting in several National Forests. The Corps of Engineers refused to grant some dredge and fill permits in order to protect ecological and esthetic values.

With these introductory and explanatory remarks about impact statements completed, the remainder of the discussion should cover their effect on pesticide programs of the various Federal Agencies.

The Environmental Protection Agency has been involved almost continually since its beginning on December 2, 1970, in reviewing impact statements on pesticide programs. The first studied was the USDA program for the control of the imported fire ant with Mirex and the correspondence came out at about the same time (March, 1971) as our first policy statements on DDT, aldrin and dieldrin, 2,4,5-T, and other controversial pesticides. The second one of importance was the USDA gypsy moth control program. Our responses were highly critical of the conclusions on environmental impact derived from the information given in the statement. However, it was not published until months after the actual spraying so it just drew praise from the environmental organizations and served as an indicator of the position the Agency might adopt for future programs.

The EPA Regions did review several Corps of Engineer programs for the clearance of aquatic vegetation from navigable waters by the use of herbicides, usually 2,4-D. However, they drew little notice.

In November 1971, the Office of Pesticides Programs was designated the principal reviewer for all impact statements involving pesticides. A system was devised so that the EPA's comments would reflect the total environmental responsibilities of the Agency. The comments should strive to stimulate appropriate consideration of primary and secondary environmental effects by other Federal Agencies in their decision-making process. Comments should stress fundamental of fact or policy and should be of a constructive nature, suggesting, where possible, not only what should be improved, but also how.

EPA comments are organized into one of the following categories:

> Category 1. General Agreement Lack of Objections. The Agency has no objections to the proposed action as described, suggests only minor changes, or has no comments.
>
> Category 2. Inadequate Information. The Agency feels that the statement does not contain information to assess fully the impact of the proposed project. The Agency's comments will request more information about the potential hazards addressed in the statement, or ask that a potential hazard not addressed be included.

Category 3. Inadequate Information/Objection Raised. The Agency feels that the statement does not contain adequate information to assess fully the environmental impact. However, from the information submitted, we are able to ascertain that revisions will have to be made.

Category 4. Major Changes Necessary. The Agency believes the proposed action needs major revisions to adequately protect the environment.

Category 5. Unsatisfactory. The Agency believes the action is unsatisfactory because of its potentially harmful effect. Furthermore, the safeguards which might be utilized may not afford protection from the hazards arising from this action. The Agency recommends that alternatives to the action be analyzed further (including the possibility of no action at all).

The Office of Pesticides Programs has served as the principal reviewer on the following environmental impact statements:

Federal State Cooperative 1975 Gypsy Moth Suppression Program. Treatment of 300,000 acres in Alabama, Connecticut, Delaware, Maine, Maryland, Massachusetts, New Hampshire, New Jersey, New York, North Carolina, Vermont, Virginia, and Wisconsin with carbaryl. There may be a temporary reduction of certain beneficial insects and arthropods.

Soil Inhabiting Insects Program. Application of chlordane at 35 high-risk locations (airports, military installations) in 21 states to prevent long-distance artificial spread of plant pests.

Cooperative Diapause Control Program for Boll Weevils in Texas and Mexico. The statement describes present and future plans for controlling the spread of boll weevils in the Texas High Plains. It recommends the use of the insecticide aldicarb, in order to reduce the use of malathion. Aldicarb is highly toxic and will be applied only to rows in the margins of selected fields near ideal overwintering quarters. Some studies have been done to determine aldicarb's effect on insects, animals, etc.

Cooperative Federal-State (North Carolina and South Carolina) Regulatory Program for Witchweed, Involving the use of 2,4-D and paraquat. The objective of this program is to retard the spread of main areas infested by witchweed plants and to eradicate small infested areas isolated from the main areas.

U.S. Air Force. Disposition of Orange Herbicide (a defoliant) by Commercial Incineration at Deer Park, Harris County, Texas, and Sauget, Illinois. The empty drums will be decontaminated;

crushed, and buried in a landfill pit. This statement involves the discharge of 44.68 million pounds of CO_2 into the atmosphere, and the liquid water-salt effluent from HCl acid scrubbing action will discharge 12.4 million pounds of NaCl into a public stream.

Imported Fire Ant Cooperative Federal-State Control and Regulatory Program. Aerial application of Mirex to 20 million acres in Alabama, Arkansas, Florida, Georgia, Louisiana, Mississippi, North Carolina, South Carolina, and Texas.

Federal-State Cooperative Spruce Budworm Suppression Project, 1972, Aroostook and Penobscot Counties, Maine. Chemical treatment of 600,000 acres of State and private commercial woodland with Zectran.

1972 Siskiyou National Forest Herbicide Program (Josephine, Curry, and Coos Counties, Oregon). The selected herbicides 2,4-D, 2,4,5-T, or atrazine will be applied to 213 separate tracts totalling 11,058 acres during one or two application seasons. Reduction of vegetative competition to increase survival of newly planted conifer seedlings is the treatment object.

Federal-State Cooperative Grasshopper Control on Western Rangeland. Chemical treatment of 2.5 million acres of rangeland with malathion.

Proposed Animal Damage Control Act of 1972. Repeal of the Act of March 2, 1931. Restrict the use of poisons for killing predatory animals on Federal lands. Role of the Department of the Interior in animal damage control will consist of research, extension, regulation of animal damage control on Interior lands, and administer a phase-out, 3-year grant-in-aid program to the States for predatory animal control. The proposal is national in scope, but largely affects range lands in the West.

Herbicide Control of Big Sage Brush with 2,4-D (Beaverhead National Forest, Madison and Beaverhead Counties, Montana). Specific control areas are being planned and designated on the ground by the Ranger with the assistance of specialists in ecology, wildlife biology, soil science, and range science. The chemical 2,4-D may have some effect on animal organisms and nontarget plants.

Removal of Canada Plum to Control Green Peach Aphid in Aroostook and Penobscot Counties, Maine. The overwintering host plant (Canada Plum) of the green peach aphid will be removed by chemical and mechanical methods from certain test areas. The trees will be cut and the stumps painted with ammonium sulfamate. Trees left uncut will be sprayed with the insecticide demeton.

Department of Justice and Indiana State Government treated 85 sq. miles of [illegible] and [illegible] miles of ditch and shoulder road with 2,4-D in Putnam County for eradication of marijuana.

Demonstration of Modular Internal Spray System for spruce budworm control in [illegible] National Forest, Montana. One of the project objectives was to test the suitability of using large cargo aircraft as spray planes in forest environment.

1972 Siuslaw National Forest Herbicide Use Program. The selected herbicides -- 2,4-D, 2,4,5-T, amitrole, atrazine, picloram, and dicamba would be applied to 11367 acres to control specific types of vegetation.

We have also supplied comments to other Principal Reviewers in EPA on the following environmental impact statements:

Water Bank Program, USDA. Under the Water Bank Program, incentive payments will be made to landowners and operators for conserving waters, preserving or improving migratory waterfowl habitat and other wildlife resources.

Wheat, Feed Grain, and Cotton Set-Aside Program. The Agricultural Act of 1970 instructs the Secretary of Agriculture to provide for a set-aside of cropland if he determines that the total supply of wheat, feed grains, or cotton will likely be excessive, taking into account the need for an adequate carryover to maintain reasonable and stable supplies and prices and to meet a national emergency.

U.S. Army Engineers and Virginia Commission of Game and Inland Fisheries. Walker Dam Impoundment Aquatic Plant Control, New Kent County, Virginia. Initiate program to control infestation of elodea with diquat and potassium endothal.

Control of Eurasian Watermilfoil in TVA Reservoirs. There are two methods of controlling watermilfoil: Water level management to dry the plant by dewatering or by otherwise disturbing its habitat, and application by 2,4-D herbicide applied by boats.

National Center for Toxicological Research, Pine Bluff, Arkansas. Development of existing facilities for use by the Center with initial action to be conversion of 29,000 sq. ft. of space to animal holding and research use.

Approval of polyvinyl chloride liquor bottles. The Internal Revenue Service prepared a statement because of the solid waste disposal effect on the environment.

As you heard the great number of impact statements are generated in the Forest Service of the U.S. Department of Agriculture. In fact, USDA spent over 2 million dollars in the preparation of impact statements on pesticide programs, flood-control projects, and other projects in fiscal year 1972. This, at times agonizing appraisal caused the U.S. Department of Agriculture to amend the way it normally conducts business. The Modular Internal Spray System tests in the Lolo National Forest in Montana was originally scheduled in the Flathead Indian Reservation in western Montana but was changed during the preparation of the impact statement. Again, the Forest Service cancelled a proposed spray program with 2,4,5-T for the control of mesquite in the Coronado National Forest, Arizona, after filing copies of the draft impact statement with CEQ. In our response to the Forest Service program for using six herbicides-- 2,4-D, 2,4,5-T, amitrole, atrazine, picloram, and dicamba-- for controlling vegetative growth, we suggested that the program be limited to the use of 2,4-D, 2,4,5-T, and atrazine. The Forest Service concurred with our recommendations and modified their program accordingly.

Again in 1972, the press cast us as completely antagonistic to the gypsy moth control programs of the U.S. Department of Agriculture. Our comments led to a reduction of about 50,000 acres in the planned 195,000 acres and the development of a monitoring study on the effect of carbaryl on birds, fish, and wildlife. We feel that information from this study will help in assessing environmental damage. Additionally, the final impact statement covered the cost-benefit aspect of the spray program and some solid figures on the effect of the pest on real estate values, tourism, erosion control, and aesthetics were generated.

Our responses are not always interpreted in a way we expect. In all cases we have been, and we will continue to be, impartial in our judgments--no matter what readjustments are necessary and no matter whom our comments affect. We were very complimentary in our responses to a Forest Service program for eradication of sagebrush with 2,4-D in Beaverhead National Forest, Montana. However, the State of Montana Department of Fish and Game was highly critical of the proposed program and also of our response. An exchange of views with this State Agency has already started and we hope that out of this conflict can come mutual benefits.

The EPA publishes periodically in the Federal Register a listing of draft statements that have been reviewed and commented upon. The listing includes the Federal agency responsible for the statement, the title of the statement, and the classification of the nature of EPA's comments. Copies of these comments are available from the Office of Public Affairs, EPA, Washington, D.C. 20460.

Earlier, I mentioned that the Food and Drug Administration recently published a notice of their proposed environmental impact statement procedures. They are asking that manufacturers seeking premarketing clearance of human and animal drugs, food additives and color additives include impact analysis reports with their submissions. Then, FDA itself would act upon the reports and prepare the impact statements for the required marketing clearances. At the present time, EPA does not require such a report be filed with a petition for the registration or establishment of a tolerance for a pesticide compound.

There are signs of a back lash against the National Environmental Policy Act. Some of the antagonism springs from the uncomfortable burden of paperwork and the cost in manpower and dollars. However, the most immediate sourse of anxiety is the enthusiasm with which Federal courts have been interpreting the Act and delaying Federal programs, especially the public works projects throughout the country. It is not surprising then, that there are several bills in the Congress to exclude certain Agencies from the preparation of impact statements but it appears that this will not include the pesticide-oriented programs.

I feel that we in the Environmental Protection Agency and you in your official capacities should continue and expand our efforts in studying these proposals which affect us and this world we live on. Thank you!

USDA-APHS ENVIRONMENTAL STATEMENT

This statement was distributed by Mr. William Hoffman and students reported on their work group findings in the course.

Title of Statement: Rangeland Grasshopper Cooperative Control Program

Type of Statement: Draft (X) Final ( )

Date: February 9, 1972

Type of Action: Administrative (X) Legislative ( )

Statement:

1. Description and Background

Grasshoppers are a serious pest in the Great Plains and Mountain States. They can cause complete devastation to range and cropland during outbreaks. No year passes without significant damage occurring to rangeland in some areas. The pests compete with cattle and wildlife for grass and browse, and during severe outbreaks, feeding areas may be completely devastated. Cattle are sometimes sold early or shipped to other range areas because of grass depletion. These outbreaks can surpass the ability of individuals to handle the problem on a local or community basis. Because of this, it has been determined that federal assistance in protecting range grass and preventing severe population buildups which may lead to large-scale migrations to agricultural crops is justified and in the public interest.

Most range grasshoppers are not general feeders as are crop grasshoppers. Some of these species eat only grass, some feed on plants other than grass, and some prefer shrubs. Since as many as 20 species may be found living together, nearly all range vegetation is subject to attack despite the specialized feeding.

Even when the infestation of a range is light, with an average of six or seven grasshoppers to the square yard, the grasshopper population on each 10 acres consumes grass at about the same rate as a cow. If the grass is sparse, the amount thus destroyed may reduce the supply to the point where cattle can no longer subsist on it. During outbreaks, when there may be 30 to 60 grasshoppers to the square yard, all the grass may be destroyed.

The amount of economic damage caused by a given number of grasshoppers on a given area of range varies with changes in temperature and rainfall. During cool weather and abundant rainfall, grass is likely to be plentiful, and the amount consumed by the grasshoppers is not economically significant. In hot dry weather, however, grass is likely to be scarce, and the amount consumed is a serious matter. Also, some grasshoppers eat more in hot weather than in cool weather (9).

During the midthirties, grasshoppers were so prevalent in the Midwest that farmers and ranchers could not cope with the situation. Funds were first appropriated by the Congress in 1934 to cooperate with the States in organized grasshopper control programs. Since 1937, a program has been conducted almost annually with funds provided partly by individuals, counties, states, and the federal government in accordance with the needs as determined by an annual grasshopper survey.

In 1948, it was recommended by State officials from the States affected by the grasshopper problem that, in view of the effective insecticides available, the federal government should be relieved of the burden of assisting farmers in combatting cropland grasshoppers. It was reasoned that the cropland farmers were now able to protect their crops and crop protection as such was no longer considered a federal responsibility. Where grasshoppers originate on a particular farm, doing damage only on that farm, individual action is sufficient. However, if they are present in such numbers that movement from one farm to another could occur, community action is needed. When severe outbreaks extend over large areas, the cooperation of county, State, and federal agencies is essential to successful control. This is particularly true in range areas where some land is privately owned, some State owned, and some owned by the federal government. Only a few species attack crops, but more than 100 species feed on range vegetation. If not controlled through an organized effort, many of these will move into cropland from denuded range areas. This new policy was implemented in 1949 with the federal government furnishing only technical assistance for grasshopper control in croplands.

The objective of the present program is to control threatening economic populations in cooperation with ranchers, State, and federal agencies to protect rangeland and prevent population buildups which could spread to valuable croplands. The treatment of incipient outbreaks reduces the extensive use of pesticides should the pest be permitted to spread into other range areas or into cropland.

In 1951, the present policy was established whereby the cost of rangeland grasshopper control would be shared by individuals, States, counties, and the federal government, with the latter providing one-third of the total cost on private and State lands. Survey and control on federal lands such as those administered by the Bureau of Land Management and the Bureau of Indian Affairs, U.S. Department of the Interior; and the Forest Service, U.S. Department of Agriculture, continued to be the responsibility of the federal government. To support this effort, memoranda of understanding have been consummated between Agricultural Research Service and the Forest Service and between USDA and the USDI. Since 1951, when modern insecticides came into general use in the grasshopper program, there have been no widespread outbreaks or severe losses. With the exception of 4 years, in the late fifties and the years 1966 and 1967, the use of control materials has been greatly reduced. This has been due to the development and use of improved methods and procedures which resulted in more effective control.

Grasshoppers have been at an unusually low level for the past several years. However, during 1971, heavy population buildups occurred in several western States. In any given year, all acreage that should be treated does not receive treatment. Landowners as a rule do not favor treatment in years of light infestations, nor do they favor treatment unless damage is visible. When damage is occurring, it is generally too late to organize and prepare for a cooperative program. Too often, the rancher that was aware of the potential in March and April and not interested in a program is desirous to participate in mid-July or early August when the grasshoppers are in the adult stage and egg laying is taking place. Treatment at that time does not reduce future populations that could build up under weather conditions favorable to grasshopper development. In an ideal control program where grasshoppers are reduced to one or less per square yard, they will probably remain harmless for several years. The same area under these conditions is seldom treated more than once in 5 to 10 years.

Despite large-scale control programs in 1971, severe damage to crops in Idaho was caused by grasshopper migrations from range areas. Damage to the range grasses also occurred in Idaho, as well as in Colorado, New Mexico, Oregon, and Wyoming. If conditions are favorable for grasshopper development in the spring of 1972, serious outbreaks can be expected.

If control programs are required in the infested States, the insecticide malathion will be applied by contract aircraft at the ultralow volume (ULV) rate of 8 fluid ounces or 0.64 pound of active ingredient per acre. The insecticide carbaryl has also been used in recent years for grasshopper control with good success. When carbaryl is the insecticide of choice, it is applied at the rate of 16 fluid ounces of 8 ounces active ingredient per acre. The choice of approved chemicals is normally mutually agreed upon by the cooperators. One application of malathion or carbaryl is required for control. It is sometimes necessary to retreat an area and apply a second application when rains occur immediately after treatment. Approximately 2 million acres of rangeland in the Western States may require treatment in 1972. The size of the areas may range from 5,000 to 400,000 or more acres. Some of the work will be done on State land and the public domain, but primarily on privately owned land.

In 1971, the actual cost of treating with malathion, including application, was about $0.60 per acre. The one-third cost to the rancher was about $0.20. However, where cropland is interspersed in with the rangeland, the grower pays the entire $0.60 per acre for the cropland treatment. Due to rising costs, the per acre cost in 1972 may be slightly higher.

Adult grasshopper surveys are conducted during the late summer and fall each year, and a map is prepared showing the areas and potential severity of infestations for the following year. The surveys are made by trained personnel making a number of stops in each county. The number and location of these stops are dependent on the nature of the infestation, the size of the county, and the supervisor's knowledge of grasshopper development during spring and summer in his area. The adult survey maps are furnished to other Federal agencies, to county agents for dispersal to farmers and ranchers, and to other interested groups. State-Federal meetings are held with the ranchers during the fall and winter months, apprising them of infestations and expected need for control the coming year. After grasshoppers begin hatching in the spring, nymphal surveys are made where economic populations were found during the adult surveys. This survey is the criterion for determining whether or not control will be necessary. Before any control is undertaken on private land, the ranchers must show an interest and cooperate in the program.

Research studies have shown that on the average, eight or more grasshopper nymphs per square yard in the fourth and fifth instar can cause economic damage. For most species, there are five instars or stages of development preceding the adult stage. This can vary with the season. In some cases, when grass is sparse, fewer grasshoppers can cause damage; and when grass growth is lush, the range can sustain higher populations without significant loss.

Statutory Basis for Conduct of Program

The Secretary of Agriculture is authorized either independently or in cooperation with authorities of the States concerned, organizations, or individuals, to carry out such methods as may be necessary to eradicate, suppress, control, or retard the spread of certain insect pests, plant diseases, and nematodes, under enabling statutory authorities granted the U.S. Department of Agriculture by the Congress. The authorities pertaining to the control of grasshoppers are listed below:

> Incipient or Emergency Control of Pests
> (Joint Resolution of April 6, 1937, as amended;
> Title 7, United States Code, Sections 148-148e.)
>
> Organic Act of the Department of Agriculture
> (Act of September 21, 1944, as amended;
> Title 7, United States Code, Section 147a.)
>
> Cooperation with State Agencies in Administration
> and Enforcement of Certain Federal Laws (Act of
> September 28, 1962; Title 7, United States Code,
> Section 450.)

Each State has basic pest control authority permitting participation in cooperative pest control programs.

2. Environmental Impact

Malathion treatments for grasshopper control have been monitored for several years. Results of these studies show that, while some beneficial insects and other nontarget organisms are affected, any damage that may occur is of limited extent and duration. In monitoring studies on fish and wildlife in Nebraska, Utah, and Wyoming on grasshopper control, it was found that technical grade malathion sprayed at the rate of 8 fluid ounces or 0.64 pound active ingredient per acre appeared safe for terrestrial and aquatic wildlife when adequate precautions are taken in sensitive areas.

Malathion was applied by aircraft to approximately 49,000 acres of scattered grassy parks throughout the Big Horn National Forest of Wyoming in August of 1965 (2). The rate was 8 fluid ounces, ULV, per acre.

Observations of relatively small trout streams containing brook and rainbow trout were made. The treatment did not affect the trout, although brain cholinesterase levels were lowered. The malathion treatment had a fairly severe effect on fish food organisms, although some types of organisms apparently remained unaffected. Similar studies were conducted in July and August, 1966. These were conducted to obtain additional information on the effects of ULV malathion on fish and fish food organisms. Fairly large streams contained good trout populations. On the Wind River Indian Reservation of Wyoming in August 1966 (3), the study indicated slight effects on fish and other aquatic life. Bottom animal samples indicated some reduction in fish food organisms, but numbers were adequate for repopulation. Whenever possible, stream coverage was made transverse to the stream flow to minimize effects on aquatic life. However, in fast-flowing streams where the line of flight was parallel to stream flow, the effects on aquatic life were not observed to be any more significant than when the line of flight was flown across the stream. No fish mortality was observed, either in unconfined wild fish or in hatchery fish confined to live boxes in the Wyoming tests.

On the Dixie National Forest study in Utah, July 1966 (4), the most noticeable effect of the spray application on aquatic life was the mortality of about eight, 3- to 14-inch brook trout. Most of the mortality was confined to areas where definite overlapping of the swath patterns was observed. The spray patterns were made mostly parallel to the east fork of the Sevier River, a very slow-moving, meandering meadow stream with many wide, shallow pools. Brain cholinesterase levels in samples of these dead fish were near zero. All mortality occurred in unconfined wild fish, with no mortality of fish confined in live boxes in the same area. Apparently, wild unconfined fish obtained additional chemical by feeding on dead insects. Observations, however, indicated a fairly good fish population remaining in most areas after the spray application. While no specific studies were conducted, crew members reported they observed no adverse effects or behavioral changes in any forms of wildlife.

Similar results to the Wyoming tests were obtained from a study on the Crow Indian Reservation in Montana in July of 1966 (7). Malathion was applied at 8 fluid ounces of ULV or 0.64 pound active ingredient per acre. No dead fish were observed in the stream sampled or in live boxes. The most striking effect of the treatment was the kill of aquatic organisms, although quantitative bottom samples were not greatly different. The monitoring study that was conducted in northwestern Nebraska in 1964 (13) indicated that small mammals did not appear to be affected by the treatment. Other studies, such as the cooperative study performed by Marston (8) and Pruess and Raun (10,11,12) indicated no long-lasting detrimental effects on nontarget arthropod fauna.

Ultralow volume malathion is toxic to bees when applied while plants are in bloom and the flowers are visited by working bees (5, 6). Precautions are taken by notifying beekeepers of malathion applications where rangeland treatments are made in close proximity to bee yards. This permits moving the hives when possible and feasible during the most critical periods. When the colonies cannot be moved, they are marked by a flag to prevent spraying directly over them if possible. It also has been found that covering the hives with wet burlap at the proper time reduces the damage that may otherwise be done (8).

Guidelines are furnished to the federal supervisors for their use when the cooperative State-federal-rancher control program is organized. Safety to bees is discussed at all planned conferences with cooperators, including the ranchers, and precautions will be included in the work plans. Precautionary efforts are not always effective, however, in handling this difficult problem. This is demonstrated by the fact that several beekeepers filed claims for bee losses under the Agricultural Act of 1970, Public Law 91-524, Statute 1362, November 30, 1970.

The grasshopper programs are under the direction of trained federal personnel, including skilled pilots, experienced in the application and use of pesticides. The contract pilots will be experienced, and in most western States, a State applicator's license is required. However, all pilots and aircraft meet Federal Aviation Agency requirements and are checked by USDA pilots before the work is started. Boundaries of treatment areas are easily identifiable from the air. Special precautions will be taken when spraying over water and other sensitive areas.

3. Favorable Environmental Effects

The objective of this program is to control threatening economic populations in cooperation with ranchers, State, and federal agencies to protect rangeland and prevent population buildups which could spread to valuable croplands. The treatment of incipient outbreaks reduces the extensive use of pesticides should the pest be permitted to spread into other range areas or into cropland. Over the years, much research has been done on this pest, not on just a single species, but on a number of different species native to the western United States where most damage has occurred. The research has included biology of the pest, life history and habits, including flight movements, damage done to various crops by different species, and continued studies on control. It has been found by research that if incipient outbreaks of certain species are left to go unchecked, severe outbreaks can occur. This has been borne out by the history of the development of severe infestations.

The program as it has been designed and conducted since 1951 has been successful in preventing widespread catastrophic outbreaks which formerly occurred. With the exception of 2 years, 1957 and 1958, the use of control materials has declined. This has been due to intensive survey procedures in finding incipient infestations early and treating them before they build up to unmanageable proportions, plus the fact that we have effective control materials and efficient methods of application. The grasshopper inflicts damage beyond yield losses to rangeland vegetation.

Losses under Present Program

Under this program, annual losses to grasshoppers in the western and midwestern States are as follows:

Crops	Millions of Dollars	Rangeland	Millions of Dollars
Yield losses	$18,919	Yield losses	$4,397
Control cost	.017	Control cost	.081
	$18,936		$4,478

GRAND TOTAL: $23,414

Crop losses from bordering range areas were estimated from information furnished by survey entomologists. These estimated losses of nine crops were then extended to the 18 western States that routinely have native grasshopper populations. Control costs on these crops were estimated to include insecticide treatments to an average of 200,000 acres annually at a cost of $0.085 per acre. Rangeland yield losses were estimated from known consumption of rangeland vegetation by grasshoppers. This consumption of herbage and browse was converted to dollars using the average values of $3.50 per acre with an average per acre production of 0.688 tons of herbage and browse, which were obtained from U.S. Forest Service and represent weighted average values for the 226.9 million acreage of Great Plains ecosystem found throughout the western United States. Control costs on rangeland in the western States were obtained from program records and represent the actual cost of the grasshopper program to the ranchers.

Losses with No Program

Without the program, the estimated annual losses to grasshoppers are as follows:

Crops	Millions of Dollars	Rangeland	Millions of Dollars
Yield losses	$ 99,563	Yield losses	$17,962
Control cost	.594	Rehabilitation of rangeland	5,475
	$100,157	Control cost	.830
			$24,267

GRAND TOTAL: $124,424

Without a cooperative grasshopper program, populations on the public land would not be controlled. Presently, 23 percent of infested rangeland is on the public domain. Those populations on public land would overflow and move onto range and cropland. The lack of subsidy (in form of cost sharing on a one-third basis, rancher, State, and Federal) would discourage the ranchers from treating rangeland until or unless populations were obviously threatening. Thus, without the cooperative program, grasshopper populations would be more dense and would infest a greater number of acres. These populations also would overflow and damage cropland. The estimated losses without the cooperative program were based on available information and represent the best knowledge available. Assuming that the residual life of the cooperative program is 14 years and that the program is discontinued next year, the accumulated discounted (10 percent interest) benefits and costs of the cooperative grasshopper program are estimated at $600.752/$12.442 million for a benefit cost ratio of 48:1.

This program, by the strategic use of insecticides on rangeland, is eliminating the need by the rancher and farmer to employ greater quantities of insecticides to protect his rangeland and cropland. The acreage treated under this program averaged 0.669 million annually for the past 10 years. Without a cooperative program, the rangeland acreage that would be treated by the rancher is estimated at about one million acres annually. Cropland acreage treated for grasshoppers would rise from 200,000 to 675,000 annually. In terms of insecticide consumption, the benefit of the cooperative grasshopper program is estimated at 6.20 million pounds of malathion annually. In addition, it is estimated that an average of 300,000 acres of rangeland would be denuded annually by grasshoppers. The impact of this effect on the environment, which this program is preventing, has not been estimated.

4. Adverse Environmental Effects Which Cannot be Avoided

The adverse environmental effects which cannot be avoided consist of the impact of the insecticide malathion on the ecosystem when used to achieve the program objective. The selective acreage where malathion will be sprayed and the low rate of application poses no known adverse long-term effect on the environment. Malathion at the rate used for grasshopper control has a residual toxicity in the field of not more than 7 days.

5. Alternative to the Proposed Action

Grasshoppers may be controlled by these principal means: 1) Natural enemies, 2) cultural, and 3) chemical. Natural enemies are present whenever grasshoppers are found. However, during grasshopper outbreaks, the buildup of natural enemies lags behind grasshopper population buildups and seldom becomes a significant factor in sufficiently preventing economic losses. Research has been unsuccessful in harnessing biological agents for control of grasshoppers. Cultural controls provide a valuable supplement to chemical control in cropland areas for certain species of grasshoppers. However, cultural control is unsound economically and ecologically for control of rangeland grasshoppers.

Chemical control is judged to be the only presently available method for economically controlling grasshoppers to achieve this program's objective. During the early days of this program, insecticides were used extensively in baits. In the early 1950s, aerial application of modern insecticides significantly increased man's ability to control grasshoppers, covering larger areas more quickly with less material at a lower cost. The use of these modern insecticides has permitted reducing the average population density of grasshoppers throughout the western States to the extent that outbreaks, which were common before 1950, have not occurred since. Crop losses from grasshoppers are now nearly insignificant (estimated at 0.57 percent of the farm value of host crops). The program originated from farmers seeking relief from crop damage by grasshoppers.

6. Relationship between Local Short-Term Uses of Man's Environment

The short-term objective of this program is to control rangeland grasshoppers. The long-range objective is to protect crops from rangeland grasshoppers. Sacrifices on the short-term objective will lead to an increase in losses outlined previously in this statement for "no program." In essence, there exists a balance between man's use of the land in the western States for the production of food and fiber and the use of this same land by other elements of the ecosystem. Grasshoppers are a major competitor of man for the use of this land. The cooperative grasshopper control program is believed to be the most effective method of swinging this balance in favor of man.

The present cooperative grasshopper control program reflects the public's commitment to use the resources of the western States for the production of food and fiber for mankind.

7. Irreversible of Irretrievable Commitments of Resources

The effect of malathion as used in this program in the western States is temporary and transient. It is believed that, without a cooperative grasshopper program, the balance between man and grasshoppers that existed prior to this program would be reestablished in about 10 years.

8. Consultation and Review with Federal, State, and Local Agencies

The rangeland grasshopper control program on private lands is a cooperative undertaking jointly planned and financed with the States and ranchers in the infested areas. State and rancher meetings are held during the winter and early spring months to determine the need for a control program. The need is based on the adult surveys made in the fall and verified with nymphal surveys made after the hatch begins in the spring. Often bad weather at the time of hatch can influence the populations to the point where control will not be required. Memoranda of understanding are in effect in every State where control may be required. These are supplemented by a detailed work plan for each program in a State and are approved and agreed to by the cooperators.

This program has been reviewed each year and approved by the Federal Working Group on Pest Management responsible to the Council on Environmental Quality. Personnel of the Plant Protection and Quarantine Programs of the Animal and Plant Health Service contact responsible Federal and State representatives of conservation and research agencies such as the Fish and Wildlife Service to notify them of any anticipated control program and encourage monitoring activities where applicable.

Control programs on federal lands are the responsibility of the Plant Protection and Quarantine Programs, APHS. Memoranda of understanding have been consummated with the Forest Service and the Department of the Interior. These memoranda of understanding provide that appropriate supervisors in the cooperating agencies will be furnished information on the following:

1. Recommendation of control needs.
2. Insecticide or insecticides recommended for control operations.
3. Assessment of the proposed and alternate insecticide formulation and application practices upon:
    a. Wildlife, both game and nongame species.
    b. Contamination of forage, water, and other resources.
    c. Comparative costs.
4. Damage potential to forest of conservation land systems and nearby lands if no control action is taken.

As circumstances warrant, Plant Protection supervisors meet with representatives of the other Federal agencies and private landowners with an interest in grasshopper control.

A working copy of this statement was circulated for review within the Department. Comments and suggestions received were considered and where applicable, have been incorporated into the draft. Any problems, objections, or suggestions received from other Federal, State, and local agencies and from private organizations and individuals will be discussed in the final statement.

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POLYCHLORINATED BIPHENYLS: AN INDUSTRIAL POLLUTANT

Richard E. Johnson

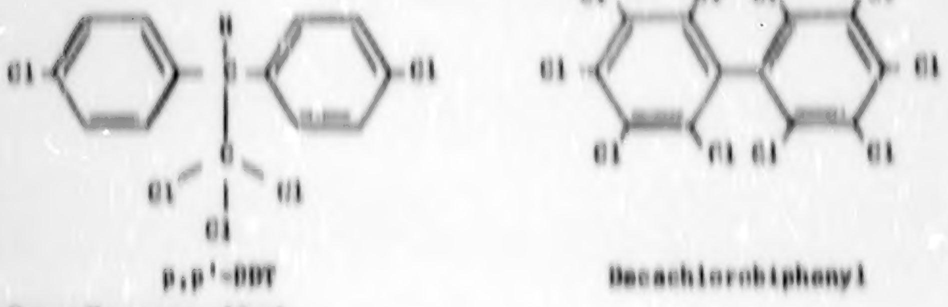
The polychlorinated biphenyls (PCBs) are a relatively new class of environmental pollutant of worldwide distribution causing widespread concern to pesticide chemists, ecologists, and, more recently, to governmental agencies both here in the United States and abroad. In the U.S., they are produced solely by the Monsanto Chemical Co. and marketed under the trade name Aroclor. The PCBs are also manufactured in Japan under the trade name Kanechlor, in Germany under the name Clophen, in France under the name Phenoclor and in the U.S.S.R. Monsanto has released production figures from 1960 through half of 1971 indicating a total production of 353,000 tons (1). Of this amount, about 42,000 tons were exported. Since the PCBs have been in widespread use since 1930, total production just in the U.S. must be staggering. Worldwide, production has been conservatively estimated at about 100,000,000 lbs. annually. Monsanto markets PCBs as products ranging from 21 to 68 percent chlorine by weight. For example, Aroclor 1254 represents a polychlorinated biphenyl formulation containing 54 percent chlorine by weight. The first two digits represent biphenyl. Figure 1 shows the chemical similarity of decachlorobiphenyl, the most highly chlorinated biphenyl, to another controversial compound, p,p'-DDT.

Since PCBs are manufactured by the direct chlorination of biphenyl molecules, there are theoretically about 210 compounds and isomers possible. Of this number about 102 are probably depending on the degree of chlorination and the positions of the chlorine atoms on the rings. This fact considerably complicates the picture since we are not dealing with a single chemical compound.

The fact that the polychlorinated biphenyls have not been found in the environment until recently is somewhat of a mystery. However, in contrast to DDT, they never were deliberately distributed into the environment and thus no one was really looking for them in environmental media. Secondly, analytical instrumentation to positively identify them were not readily available until the past few years.

Figure 1

Chemical structure of p,p'-DDT and decachlorobiphenyl



p,p'-DDT

Decachlorobiphenyl

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These chemicals were first discovered as environmental contaminants in Sweden in 1966 (2) and in the U.S. the following year. It is interesting to note the more than 35 year interval between their initial industrial use and their first reported environmental occurrence.

Why are they now widespread in the environment? Table 1 presents a brief summary of the environmental occurrence of PCBs. This table needs constant revision as the literature on PCBs is becoming very abundant.

Table 1

Environmental occurrence of PCBs

England = Rain water
Japan = River water
Scotland = Brown seals
Netherlands = Mussels
Antarctica = Adelie penguin eggs
Panama = Brown pelican eggs
Baltic Sea = Cod
Sweden = Fish and marine birds
United States = Human hair and adipose tissue, bald eagles, sewerage
California = Peregrine falcon, fish (anchovy, shiner perch, tuna, English sole, Jack mackeral, hake)
Colorado = Human milk, digested sewerage sludge, river water

It is apparent that, for the most part, those areas experiencing residues of PCBs are the more industrialized nations. However, once residues appear in rivers and oceans their distribution can be widely broadened. Through 1969, there were approximately 35 general uses of the PCBs. Table 2 presents a resume of these uses (some uses are combined) to illustrate how broad their industrial application base has become in or on various commodities.

Table 2

Uses of polychlorinated biphenyls

Flame proofing of cellulose fabrics
Electrical insulating fluids
High-vacuum systems
Anodized aluminum surfaces
Plasticizers in plastics
Hydraulic fluids
Insulation products and insulating liquids
Heat transfer exchange fluids
Lubricating compounds
Plastic laminating material and adhesives
Corrosion inhibitors and coatings
Extreme high pressure lubricants
Antistatic coatings for plastics
Thermographic copy paper and solutions
Rubber and dyes
Lacquer and polishes
Permanent stencils
Concrete coatings
Paints, waxes and varnish coatings
Cutting oils
Metal decorating and printers ink
Graphite mixtures
Grease-mashing materials
Asphaltic materials
Putties and cellophane
Sealing materials
Flame retardant coatings
Fingernail polish

In fairness to Monsanto, they have voluntarily agreed to limit the use of PCBs to sealed systems in response to mounting evidence that PCBs have widespread distribution and are a potential hazard.

One might again ask the question of why they are widespread in the environment? Table 3 lists some of the physical properties of PCBs, properties which have insured their environmental persistance.

Table 3

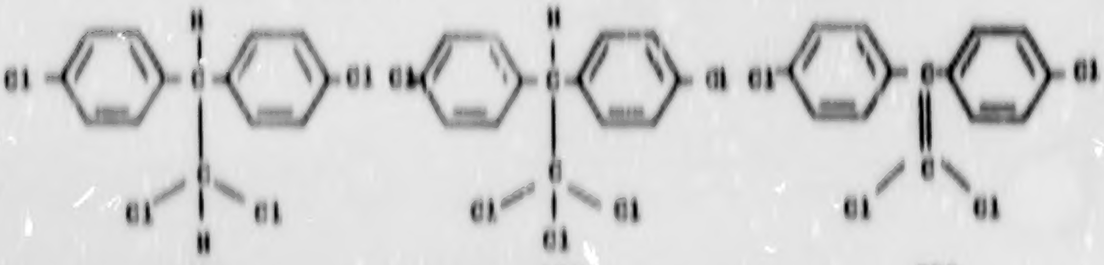
Physical properties of polychlorinated biphenyls

1. Low vapor pressure (high boiling point)
2. Low water solubility (high lipid solubility)
3. High dielectric constant (non-conducting)
4. High thermal stability (to 870°C.)
5. Permanently thermoplastic
6. High resistance to acids, alkalies, and other corrosive chemicals
7. High resistance to oxidation and environmental breakdown
8. Vary from oily liquids to white crystals and hard transparent resins.

Figure 2 lists the structure of DDT and two of its metabolites, DDE and TDE. Note that metabolism is limited to the two-carbon chain between the phenyl groups. The PCBs lack this "handle" for metabolism and, partially for this reason, are considerably more resistant to breakdown than is DDT.

Figure 2

Structure of p,p'-DDT and its metabolites DDE and TDE



TDE DDT DDE

When gas-liquid chromatography (GLC) replaced traditional chemical methods, such as colorimetry, in the early 1960's in the pesticide analytical laboratory, it became apparent that tissues of certain animals - particularly the fish eaters - contained "unknown" peaks in addition to the known pesticides. Using GLC with detectors relatively specific for chlorine containing compounds, it was known that they were chlorinated chemicals. These unknowns had been the subject of much discussion and concern although little had been written about them.

On the GLC tracings, the PCBs, for example Aroclor 1254, have 16 or more peaks, some of which overlap those of the DDT group and coincide with other insecticides as well. Figure 3 illustrates typical GLC chromatograms of Aroclor 1254 in the center, an extract of digested sewage sludge on the top and a mixture of the two on the bottom. It is apparent that a chlorinated hydrocarbon mixture very similar, if not identical to Aroclor 1254 is present in the sewage sludge. One can readily see from the complexity of the chromatrogram for a PCB how confusion can and has resulted in both identifying residues and quantifying residue data when both PCBs and DDT or other pesticides are present in the same extract.

One might next ask the question of how do the PCBs get into environmental media. Table 4 presents some of the possible routes. Very little is currently known about this aspect of the PCBs.

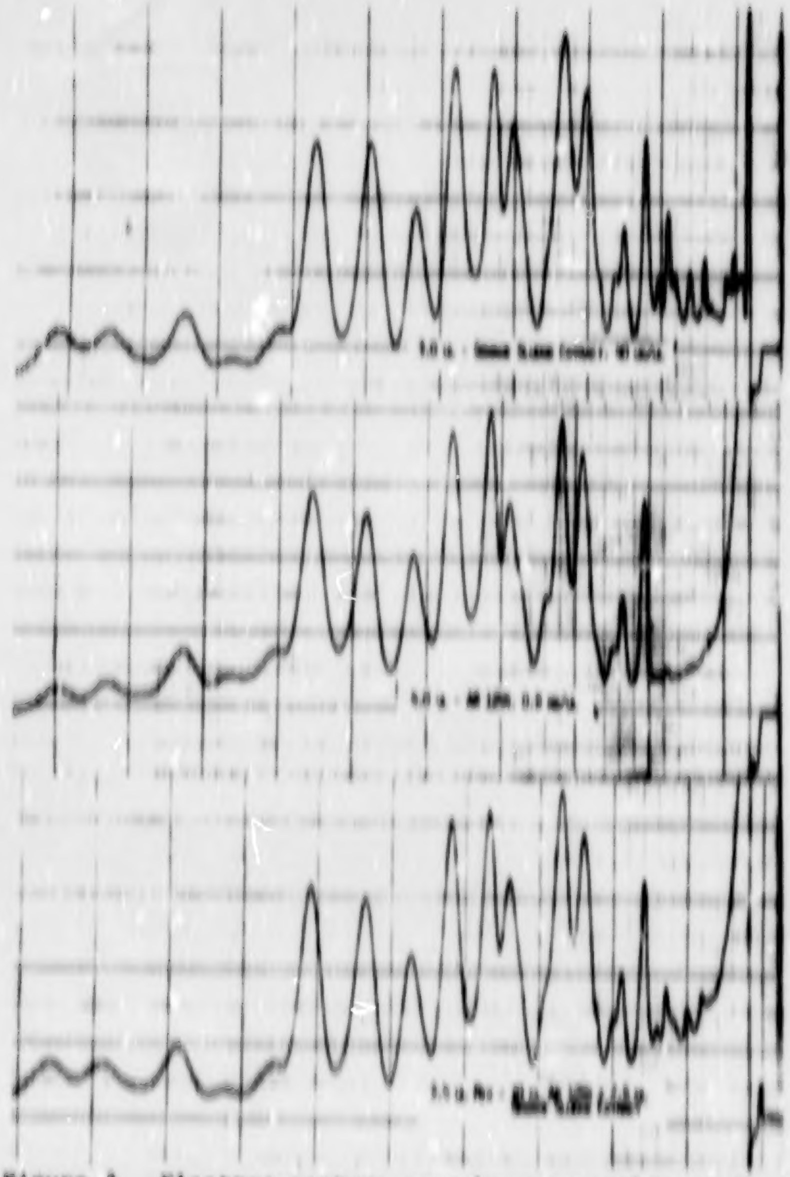


Figure 3. Electron-capture gas chromatographic analysis of a digested sewage sludge extract, Aroclor 1254, and a mixture of the two.

Table 4

Possible routes of PCBs into the environment

1. Incineration of waste materials
2. Accidental leaks of equipment (e.g. heat transfer systems)
3. Weathering or friction-wearing of the many materials having PCB's as an ingredient
4. Sewage disposal systems
5. Interaction with food products in their uses as an ingredient in substances like plastics and paints.

My laboratory has been involved in a number of studies with PCBs. One study involved measuring the volatilization of Aroclor 1254 and 1260. Even though their vapor pressures are very low, about 5% was volatilized after one week from a deposit and slightly more from an aqueous suspension, both under ambient laboratory conditions. It was evident that the lower chlorinated compounds volatilized more rapidly.

This laboratory has found, as indicated earlier, PCBs in digested sewage sludge from Fort Collins. We have also found PCBs in sludge from a number of other locales in Colorado. However, GLC evidence is not conclusive in identifying a compound.

More information was gained by examining both the extract and Aroclor 1254 on a microcoulometric gas chromatograph, a GLC with a detector specific for chlorine. This data also indicated that the extract contained PCBs essentially identical to Aroclor 1254. To get structural proof that the unknown is indeed Aroclor 1254, infrared spectra were obtained as shown in Figure 4. The infrared spectrum of the sewage sludge extract is consistent with the known Aroclor 1254 spectrum. Note the aromatic substitution absorption bands between 800 and 900 cm^{-1} and the consistent "fingerprint" area of 1000-1200 cm^{-1}.

The next aspect of the work was the separation and identification of sewage sludge components by GLC coupled with a mass spectrometer (AEI Model MS-12). A direct probe analysis was made with both the extract and Aroclor 1254, using a concentrated sludge extract whose physical appearance was similar to 1254, being a clear and very viscous liquid. The results indicated that the highest isomer in both samples had a mass of 392 indicating a heptachlorobiphenyl. The spectra obtained showed the intense fragment ions produced by the consecutive loss of chlorine atoms from the parent ion.

The data from the GLC coupled to the mass spectrometer gave proof that the sewage sludge extract contained 4 tetrachloro-, 3 pentachloro-, 4 hexachloro- and 2 heptachlorobiphenyl isomers. The peaks from the sludge extract were identical in mass to those of Aroclor 1254 confirming the presence of PCBs in the sludge.

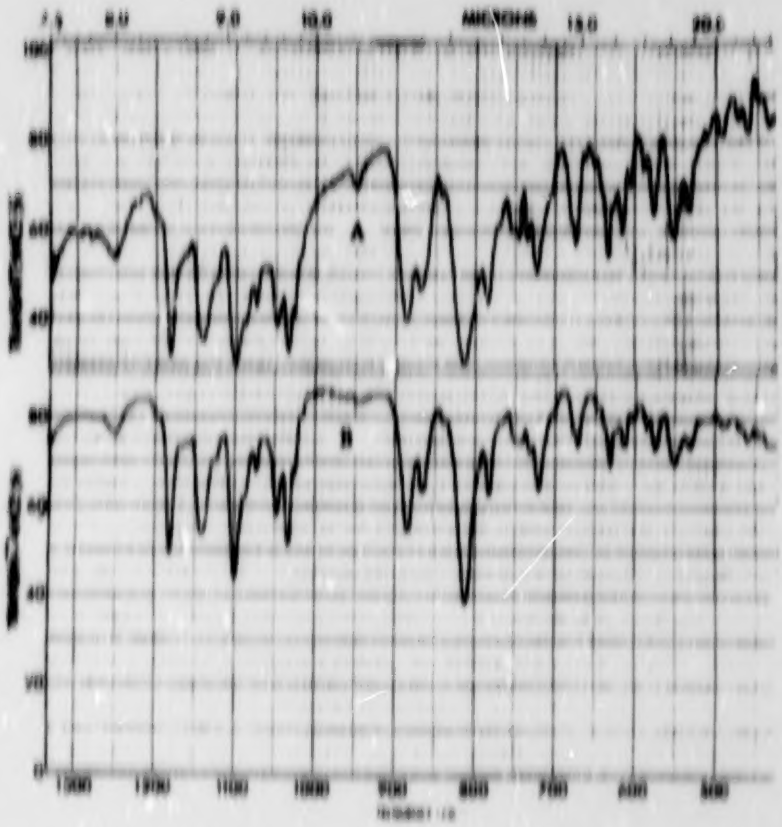


Figure 4. Infra-red spectra of Aroclor 1254 (A) and a digested sewage sludge extract (B).

We also have additional supporting GLC data using a Coulson electrolytic conductivity detector, thin-layer chromatographic data and spectrophosphorimetric data.

Recent work, on the metabolism of Aroclor 1254 and a number of known PCB isomers, in soil indicates no breakdown in a system where DDT is almost 100% metabolized. A recent study showed that PCBs are readily absorbed into the root systems of certain plants. We have also confirmed the presence of PCBs in water and sediment downstream but not upstream from a local sewage plant outfall.

There are a number of research needs that should be investigated to clarify the many questions remaining. Such needs are to determine the source of pollution, the extent of biological concentration, toxicity studies, transport mechanisms and metabolism in various biota and ecosystems.

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EPIDEMIOLOGY OF ANIMAL POISONINGS OTHER THAN HEAVY METAL POISONINGS

Frederick W. Oehme

Epidemiology is defined as the science of the prevalence of a disease or diseases in a community. In human medicine this refers essentially to contagious or infectious diseases and their spread throughout a population. In veterinary medicine, infectious diseases are only a portion of the disease problem. Disorders produced by chemicals (poisonings) and improper management are at least equally important, and since animals are almost entirely dependent upon man for providing the essentials of proper nutrition, housing, and their total environment, animal diseases produced by chemicals have relatively more importance in veterinary medicine than in human medicine.

Not only is the relative incidence of diseases more variable in domestic animals, but pets and livestock are different beasts than man. Their anatomical differences are obvious. Coupled with differences in structure are physiological differences in organ size and function, and in capacity and limitations of tissue activity. Differences are also found when the biochemistry of individual tissues and animals are examined. Enzyme capabilities in the digestive tracts, metabolism and biotransformation systems in the liver, and transport and other enzymatic processes throughout the body differ markedly from species to species and between domestic animals and man. Indeed, the occurrence of these variations is the basis for the entire group of comparative sciences.[13,20]

Of equal or more disease-producing significance than anatomical, physiological, and biochemical differences are the effects of domestication and the resulting characteristics that our animal population have developed through years of close affiliation with man. Domestic animals have essentially become creatures of habit and have maintained the specific feeding, grazing, watering, and daily routine habits that man has imposed. Irregularities in diet and routine produced by mismanagement leave the animals with little alternative. Due to their complete domestication, animals have become entirely dependent upon man for their total care and well-being. They thus have become subject to man's whims, his attempts to become efficient or "cut corners", and also to his stupidity and errors. The domestic animal is forced to feed and be housed where man wills. Feed, water, and therapeutic or prophylactic application of chemicals make animals susceptible to errors in dosages, feed ingredients, faulty applications of chemicals, and general mismanagement, with the potential for toxicity always present.

With such a wide variety of potential sources of errors due to animals' complete lack of independence and total acceptance of their environment, it is common to find chemical poisonings occurring under wide circumstances. The epidemiological pattern of animal poisonings is closely related to man's ability to properly manage his pets and livestock. The following are examples of some of the common animal poisonings and reflect the variety and importance of this area to the veterinary practitioner.

Drugs and Household Products

Errors in the choice of a therapeutic compound, in its route or mechanism of application, and in its dosage are common lapses of judgment that result in poisoning. The faulty selection of a worming preparation, the administration of a new chemical by an other-than-recommended route of administration, and the overdosage of an anesthetic are dangerous and frequently fatal errors. The mistaking of propylene glycol for mineral oil and the administration of 1 gallon of the wrong material to horses has recently resulted in a characteristic toxic reaction.[12] Not only are such errors subject to frank poisoning, but adverse reactions and the multitude of possible acute and chronic variations may also occur.

Household chemicals are a popular source for human poisonings. This is also becoming common in veterinary medicine due to the increased number and diversity of chemicals found and used in the home. Aspirin, vitamin and mineral pills, birth control tablets, marijuana and drugs of abuse, disinfectants, and germicides are only a few of such hazardous materials.[11,12] Because types of household pets vary from birds through rodents to dogs and cats, a wide variation in toxic response is possible. Hence, owners used to exposing their previous pets to the usual household chemicals, may find toxicities developing upon the introduction of a new species of household animal. The phenolic disinfectants are one group of commercial compound for which this species variation is potentially of importance.

Figure 1 illustrates the normal phenolic components of urine from four different animals. The wide variation in phenol metabolites is obvious and reflects the variability in ability to detoxify phenolic materials. Figure 2 is the plasma disappearance of radioactive phenol in cats, dogs, pigs, and goats following the intravenous administration of identical single doses. It can readily be seen that the pig and goat demonstrate the ability to rapidly detoxify and eliminate phenol from the blood. This represents not only liver metabolism, but also excretion, primarily via the kidneys. The dog is seen to be intermediate in its rate of plasma clearance, while the cat has a relatively slow capability of removing the toxic phenol from the plasma. As long as the plasma level of the toxic chemical remains high, toxicity is a potential problem. It would further be expected that pigs and goats have little potential for phenol poisoning, dogs would have an average potential for toxicity, and cats would be presented with a greater hazard from exposure to phenolic materials.

If dogs and cats are then exposed to similar doses of phenolic compounds and their survival time measured, it can be seen that cats respond with greater toxicity than dogs (Figure 3). Although two different dose ranges are used, dogs had the ability to survive the phenol challenge in both cases while cats, on the average, died in a matter of hours. When urinary excretion of the phenolic compounds is measured, dogs are observed to be more capable of excreting the toxic material than cats (Figure 4). Thus, the capacity of dogs to metabolize and excrete the phenolic compounds is apparently related to that species ability to withstand doses lethal to cats. Although this species difference has been experimentally demonstrated for a wide variety of household chemicals (aspirin, cleaning fluids, petroleum products, insecticides, disinfectants), the phenolic dosages used to document this effect were greatly in excess of the household exposure to which pets are subjected under usual conditions of household use.

Nevertheless, were it not for man's faulty judgment in applying such compounds directly to and around our domestic animals, toxicities due to errors in selection of the chemical, application route, dosage, or species difference ("I think I'll bathe my cat with this new shampoo; it worked so well on my dogs last year!"), the incidence of poisoning due to drugs and household products would be greatly reduced.

Pesticides

This group of chemicals includes insecticides, rodenticides, herbicides, and fungicides; the latter two have the least potential for toxicity.[14] The newer herbicides and fungicides are in general relatively safe, and only the use of some of the older more toxic chemicals or exposure to organic solvents used to carry the chemicals in their application are likely to produce poisoning.

However, rodenticides are an important group of chemicals causing animal poisonings. Strychnine is probably the most common cause of poisonings in dogs and is frequently used to "cleanse" the neighborhood of undesirable dogs.[1,2,4,11] The fact that strychnine can be purchased under the guise of rodent bait greatly increases the hazard of poisoning. Other compounds used for rodent control are at least equally toxic, but the limited distribution of such materials as 1080 (sodium monofluoracetate), ANTU (alpha-naphthyl thiourea), phosphorus, metaldehyde, and red squill makes incidence of their toxicity much less than that of strychnine. Warfarin is a readily-available compound used to control rats and mice, but toxicity is limited by the necessity for repeated dosages to be ingested before poisoning usually occurs.

The insecticides are a much more complicated and diverse group of foreign compounds.[14,20] As causes of animal poisonings, the chlorinated hydrocarbons and the organophosphorus and carbamate materials are hazardous. Fortunately, effective treatments are available for the organophosphorus and carbamate compounds; the chlorinated hydrocarbons are less effectively treated. The widespread use of insecticides around large and small animals result in considerable hazard to these species. Poisoning usually occurs from accidental exposure via spray drift or due to the intentional application of the insecticide to control livestock or pet insects. In the latter instance, toxicity may result from improper dilution of the concentrated material or too frequent application of acceptable amounts. Both problems are diminished by users properly reading the labels of the respective products and abiding closely by the specified recommendations.

While direct toxicity to the exposed animals is a foremost concern, many of the pesticides are capable of accumulating in feed or food supplies following their application to the environment. Hence, the widescale use of insecticides to animals on pasture may result in hay or feedstuffs growing on neighboring fields developing residues of these foreign chemicals. Likewise, if proper precautions in marketing the exposed cattle are not followed, the meat and by-products from such animals may contain high residues of the applied chemicals. Such matters are of vital interest to governmental regulatory agencies and provide a significant concern in their efforts to protect not only animal, but also human health.[10]

The government's concern with the accumulation of foreign compounds in not only foods, but also the general environment, has resulted in the consideration of restrictions on the use of many of the pesticides currently in use. Foremost in such action was the recent banning of DDT from routine usage in the United States. This has been followed by other chlorinated hydrocarbons being examined as a preliminary for similar restrictions. Many of the rodenticides (strychnine, 1080, ANTU, warfarin), and some of the heavy metal materials used as herbicides and fungicides, are being re-evaluated by regulatory agencies because of their hazard and adverse environmental impact. Whether they will indeed be banned from use or only restrictions placed on their applications remains to be seen.

Fertilizers

The problem of synthetic materials, such as nitrates and ammonia, and also naturally occurring organic material, such as manure, contributing to animal poisonings may seem remote. Unfortunately, synthetic and natural fertilizers are an important cause of livestock toxicoses. The most obvious situation results from the excessive use of nitrate-containing fertilizers on crops and the accumulation of high nitrate levels in the harvested product. These nitrate concentrations in animal feeds can produce acute or chronic nitrate intoxication and widespread economic loss.[1,2,4,12,15,19] Even the application of manure to fields can result in high levels of nitrates developing in plants growing in such areas. Not only do cash crops accumulate toxic levels of nitrate, but certain weeds, such as pigweed (Amaranthus retroflexus), are capable of building up high levels of nitrate in their organic matrix when grown on soils containing concentrations of nitrates or nitrate-releasing organic matter.[1,8,12,20]

An instance of a vacant feedlot that had grown a lush crop of weeds and was used to provide green pasture for cattle illustrates this problem. Within a few hours after a large group of feeder cattle were turned into the weed-covered feedlot, the owner found 17 cattle dead and numerous others in various stages of toxicity. Even though the cattle were promptly removed from the lot, approximately one-third of the animals died from nitrate poisoning. Analysis of the weeds growing in the area revealed concentrations of nitrate as high as 4.6%.

A complicating situation is the relationship between adverse growing conditions and the accumulation of nitrates in plant materials. Although levels of nitrate may be moderate in the soils supporting grain crops (particularly corn and sorghum) or a variety of weeds, under the influence of a drought or the application of plant hormone herbicides, these commonly-grown plants may accumulate excessively high and toxic concentrations of nitrate.[7,8] The widescale losses in the middle 1950's due to livestock consuming drought-affected corn and sorghym was largely due to nitrate toxicity. The application of 2,4-D to weeds frequently permits these plants to develop transient toxic concentrations of nitrate; if consumed during this temporary phase, nitrate poisoning may result.[8]

Natural Toxins

Toxicoses due to plants are not usual in man, but livestock and pet poisonings due to a variety of poisonous plants are a significant part of veterinary toxicology.[3,5,7,8,9,12,13,16,17] The basic factor underlying most plant poisoning problems is mismanagement.[7] Livestock are frequently allowed to graze wide areas of natural range land. When weather conditions are bad, pastures may provide less forage than expected and limited plant material for animal consumption and overgrazing frequently results. Under conditions of repeated overgrazing, naturally occurring desirable pasture grasses due out and weeds, many of them poisonous, take over the range. While such weeds are normally unpalatable, hungry lives-ock may be forced to consume them and thereby become poisoned. Although most owners recognize overgrazing and the resulting hunger that results in their animals, mismanagement aggravates the loss of native pasture grasses and speeds the introduction and multiplication of poisonous plants. When owners do not provide supplemental feeding for animals on such poor pasture land, the livestock are forced to consume the noxious weeds.

A variety of poisonous plants are available to livestock and household pets and present hazards to their health. The cyanide-producing forages are the most common cause of animal sicknesses due to plants. Usually the sorghyms (Johnson grass, sudan grass, milo) are responsible for losses. In addition, arrowgrass (*Triglochin* spp.), elderberry (*Sambucus* spp.), wild cherry (*Prunus* spp.), and the pits of several common fruits (apple, peach, apricot) contain compounds with the potential of releasing cyanide upon ingestion. Toxicities usually result from ignorance on the part of owners who feed such plant materials to their animals or who throw fence-row clippings into pastures to utilize the material for forage. Adverse weather conditions and wilting frequently increase the toxic potential from this group of poisonous plants.

Halogeton (*Halogeton glomeratus*) and black greasewood (*Sarcobatus vermiculatus*) are two plants that contain high levels of oxalic acid in their plant matrix. Although these plants are only commonly found in the ranges of western states, the oxalic acid is an extremely potent toxin and upon ingestion combines with serum calcium or magnesium. The clinical situation resulting from the sudden drop in the blood level of calcium and magnesium causes large-scale acute deaths. The loss of thousands of sheep annually in the Rocky Mountain area is directly due to the ingestion of one or both of these poisonous plants. Only recently a loss of several thousand sheep in one herd resulted in the erroneous claim that the release of experimental nerve gas had caused their death. Upon detailed examination it was found that owner-error had resulted in the sheep consuming the oxalate-containing plants and the large-scale deaths. Since the calcium and magnesium oxalate salts formed are excreted in the urine and become crystalized in the kidney tubules, causing an inability to pass urine, animals that survive the acute syndrome commonly develop renal failure.

Locoweeds (*Astragalus* spp. and *Oxytropis* spp.) are other common and characteristic causes of livestock losses in the western states. The plants are not usually consumed by livestock but under adverse growing conditions and inadequate natural forage, cattle, sheep, and horses may be forced to consume them. Once livestock taste the plants they may develop a like for their flavor and will then preferentially consume them, even if other feed is supplied. Although horses and ruminants may consume locoweeds, horses are much more sensitive to the toxic principle's effects. Hence, as in one recent incident when cattle and horses were grazing the same pasture, horses develop locoweed poisoning well before cattle show even the earliest signs of intoxication. The clinical syndrome is one of weight loss and mental derangement. Animals become easily aggravated and undergo bizarre temperament changes. Deaths frequently result from self-inflicted injuries due to running through fences, falling down wells, or drowning in ponds or streams. The toxin has an affinity for the nervous system and characteristic microscopic lesions are detected in the neurons of the brain.

Selenium is a chemical found in certain types of rock and hence specific types of soils.[18] Plants growing on such soils may accumulate levels of selenium in their plant structure varying from only a few ppm to several-10,000 ppm. While any plant growing on soils containing selenium may build up low levels of this chemical, specific plants, such as poison vetch (*Astragalus* spp.) and woodyaster (*Xylorrhiza* spp.), have the ability to selectively take up and accumulate massive amounts of selenium. Some of these plants (golden-weed, *Oonopsis* spp. and princesplume, *Stanleya* spp., for example) will only grow on soils high in selenium; hence, they are called "indicator plants", since they indicate the fact that selenium is present in the soils on which they are growing. Toxicity due to selenium will produce various clinical signs, depending upon the concentration of the chemical in the consumed plants. Low levels of selenium may result in weight loss, deformed hooves and hair loss, and the birth of deformed young. Larger amounts of selenium cause liver damage. A nervous syndrome, similar to that seen with locoism, commonly develops if large amounts of selenium are ingested.

Several other poisonous plants produce such rapid death that owners may report only that the animals were found dead. Waterhemlock (*Cicuta* spp.) grows in wet areas and contains the highest toxin concentrations in chambers just above the roots. Animals become exposed by crushing the plant in the stream in which it is growing and then consuming the toxin-containing water, or they may actually consume one or more mouthfuls of the plant material. The toxin is so severe that violent muscle spasms may knock the animal off its feet and cause death within minutes. Cattle poisoned by waterhemlock have been found with walnut-size pieces of the plant still in their mouths.

Common oleander (*Nerium oleander*) is not only responsible for sudden animal losses, but has also resulted in several human fatalities. Only a small amount of the plant material or toxin-containing sap is required to produce poisoning. Cases have developed from individuals consuming meats cooked using oleander branches as spits. The toxin affects cardiac function and produces a rapid death in all species of animals.

Cocklebur (*Xanthium* spp. is a weed capable of infesting almost any barren ground. It appears in the early spring as one of the first forms of green vegetation. Since the plant is so common, it is fortunate that its most potent state is during the early growth period, but unfortunately this is just when other green vegetation is limited. Cattle and hogs may be browsing pastures when early spring rains produce cocklebur sprouting. The resulting sprouts contain high concentrations of hydroquinone, a potent liver toxin capable of producing massive liver necrosis and death within a few hours. The mature cocklebur plant is not palatable to livestock, but the burrs containing the seeds may sprout in the fall following a warm rain. Hence, poisoning may not only occur in the spring, but also in the fall when sprouting cockleburs again provide lush forage for grazing animals.

The Japanese yew (*Taxus cuspidata*) is an ornamental plant that recently has been shown to produce acute poisonings in animals consuming clippings. The plant is frequently installed around fences and animals may become exposed to clippings or may browse the plant directly through the fence. The toxic principle has not yet been fully defined, but horses and ruminants have been found dead by amazed owners following access to this plant. Even though oleander and yew are attractive plants to beautify landscapes around homes and other structures, their toxicity provide great hazards not only for pets and livestock, but also for children accustomed to placing foreign objects in their mouths.

Some of the lower members of the plant family, the fungi, are capable of producing a variety of toxins. This particular group of poisons, mycotoxins, has become a focal point for scientific investigation during the past decade.[6] Over 10,000 scientific papers have been published on aflatoxins alone.[7] This latter group of mold poisons is produced by specific strains of the fungi *Aspergillus flavus* and *Penicillium* spp. As with all fungi, a source of nourishment and proper amounts of moisture and heat must be present to support growth. Under suitable conditions, these fungi, and others capable of producing toxins, will grow and in their growth processes will produce their toxins. Hence, the toxins are products of fungal growth; the mere presence of the fungus does not necessarily indicate that its particular toxin is also present. Conversely, the toxin can be present and viable fungi may no longer exist in the sample. Mold toxins commonly develop in stored grains and on certain feedstuffs subjected to unusual weathering or storage conditions. Although moldy feed is usually grossly identifiable, the spoiled feed may be mixed into a ration or otherwise offered for livestock consumption through ignorance or by intention. Animals will usually reject extremely spoiled feed, but well-diluted feeds or rations offered hungry cattle may result in acceptance and toxicity.

Most fungal toxins affect the liver and produce lesions varying from frank necrosis to biochemical interference with enzymes or blood-clotting mechanisms. Digestive track disturbances, photosensitazation, poor feed utilization, abortions, and reproductive failures have been also associated with mycotoxin consumption.[8,12] Although cancer has never been associated with clinical instances of mycotoxicoses, the experimental production of tumors by aflatoxins in some laboratory animals has resulted in concern over the concentrations of this toxin in grains destined for human consumption. Only a few of the toxins of potential fungal origin have presently been identified. There is no doubt that this interesting area will continue to attract the attention of toxicologists, chemists, and animal scientists for some time to come.

Feed Additives

The use of chemicals added to livestock rations for the purpose of increasing feed efficiency and reducing disease is a characteristic unique to animal production. Although this practice has greatly benefited the livestock economy, the practice is not without danger. Whenever foreign compounds are added to feeds, the possibility of error and resulting animal or human hazard increases. While the presence of chemical residues in human foods and their potential contribution to adverse effects in man is of most concern to public health officials, animals directly consuming feeds containing feed additives are also likely to become grossly toxic. This may be due to improper mixing of the ration, incomplete following of feeding recommendations, faulty husbandry, or mismanagement. In these instances, acute poisonings result from livestock consuming feeds to which they are unaccustomed or because the feed contains unusually high levels of one or more toxic materials. Two such common poisonings are those produced by excessive or improper feeding of urea or salt.

The factors frequently associated with the development of urea poisoning in cattle are listed in Table 1. Urea is a protein supplement fed ruminant animals to provide an economical source of protein to the consuming individuals. The production of ammonia from the urea by the ruminal microorganisms is normally followed by incorporation of the ammonia into bacterial protein; this protein is digested in the intestinal tract of the ruminant and serves as a source of nutrient protein. Under conditions of mismanagement (Table 1), the production of ammonia becomes excessive and the rumen microorganisms are unable to utilize the ammonia in its entirety. Ammonia poisoning then results, with the onset of clinical signs within minutes and death frequently following in 1-2 hours.[1,2,4,12,14,19,20]

Salt is a necessary dietary ingredient, but in excess or in the absence of sufficient fresh water it may become toxic.[18] Toxicity occurs most commonly in swine, but also occasionally in cattle, due to a variety of management factors leading to the accumulation of sodium ion in the central nervous system and other body tissues. If the owner then discovers that water was unavailable to the animals for a period of time, and then provides unlimited access to water, the osmotic pressure produced by the sodium results in increased central nervous system pressure and an acute neurological and convulsive syndrome.[19] While the condition in cattle is somewhat more chronic and digestive signs are more prominent, the underlying cause of salt poisoning is human error resulting in poor animal husbandry practices.

Petroleum Products

A variety of oils, greases, benzenes, hydrocarbons, and other petroleum products are used on and around domestic animals. Some are employed directly on machinery to which livestock have access; several are utilized as solvents for sprays and materials applied to animals; others are formulated for application to buildings, and animals lick or otherwise contact the applied product; or animals may directly consume the petroleum products by gaining access to storage areas housing opened containers of these materials. In certain areas of the United States, oil wells and oil storage tanks provide the potential for cattle consuming the crude petroleum product. Proper care and precautions are frequently not taken to assure that animals are protected from exposure. Incomplete fencings of crude oil holdings or storage areas may permit inquisitive cattle to satisfy their curiosity. Ignorance of the potential toxicity of these products leads owners to utilize various petroleums directly on livestock as therapeutic aids.

Petroleum products produce a characteristic sequence of clinical signs. If applied to the skin, irritation and thickening commonly results. Photosensitization is frequent, especially in white-haired or light-skinned individuals. If consumed by mouth, the petroleum material produces digestive disturbances, may be inhaled with resulting pneumonia, and after several days can produce liver, kidney, and bone marrow dysfunction. Pregnant animals may abort and a poor-doing individual, continually losing weight and eventually dying, is a usual outcome. Poisoning due to petroleum products is a complicated and varied intoxication. Its occurrence could largely be prevented by owner education and by assuring that proper precautions were taken to prevent animal access to these materials.

Pollutants

The occurrence of air, water, industrial, and other pollutions are just as much animal hazards as they are for humans. A good correlation may be observed between animal and human toxicity problems caused by chemicals polluting the environment. The animal epidemiology is frequently identical to that observed for humans.

Air pollution usually results from industrial fumes being released to the atmosphere, and animals in the vicinity are exposed to sulfur and nitrogen oxides, heavy metals such as zinc and lead, hydrocarbons, and various forms of particulate matter.[20] Since most industrial plants are located in suburban and rural areas, livestock grazing surrounding pastures are increasingly likely to assume body burdens of these chemicals or to exhibit biological responses to their inhalation. Since lead from automobile fumes accumulates in the heavier portions of the air and in particulate matter, dogs are more likely to exhibit signs of lead poisoning than are the adult humans living in the same environment.

Water pollution is a special problem for rural areas utilizing streams and wells as municipal water sources. This is in contrast to larger cities that utilize upland reservoirs many miles distant from the consuming population. The sewage discharge of upstream communities and industrial complexes and agricultural enterprises (feedlots, fertilization) may result in a variety of toxic materials being present in the water used by a downstream stockman or community for drinking purposes. The same waters may enter wells supplying other farmsteads or communities.

Recent interest in nitrate concentrations in water supplies have resulted in speculation as to the potential hazard of the continuous ingestion of low-level nitrate waters.[19] Table 2 lists the percentages of samples from selected

Kansas water supplies with the observed nitrate concentrations. A significant percentage (28.5%) had levels in excess of 50 ppm, and 19% had nitrate levels in excess of 70 ppm. While the influence of nitrates on animal and human health has only partially been defined,[18] there is little doubt that the other foreign chemicals present in water supplies due to industrial and agricultural pollution are indeed capable of producing significant toxicity.[2,20]

Other pollutants can produce toxicities under circumstances that are frequently unique to the situation. Carbon monoxide is a special problem during winter weather in animals confined in tightly sealed quarters. Tractors or improperly vented heating equipment utilized within such facilities may produce lethal concentrations of this gas. Antifreeze (ethylene glycol) is a special problem for dogs and cats because of its sweet taste.[2,19,20] Consumption of this material when owners discharge radiators in garages or on the ground results in toxicity due directly to the glycol or due to glycol metabolism to oxalate which produces renal failure through the formation of calcium oxalate crystals. As with most pollution problems, prevention is a matter of education and regulation, and prophylactic efforts are immeasurably more rewarding than the treatment of poisoned individuals.

Conclusions

The variety of foreign chemicals capable of producing animal poisonings is almost infinite. As long as such potentially toxic materials exist and are utilized by livestockmen and animal owners, hazards for animals, human and domestic alike, will be a prominent concern of the health community and of toxicologists particularly. Persons responsible for animal care must be aware of the hazards associated with the use of these foreign chemicals. They must be instructed and encouraged to use good judgment in the application of these compounds on and around our animal population. Forceful and continuing education is required to assure that safe and sensible use of toxic materials is practiced. Only if the persons handling and applying these chemicals recognize their responsibility can their use be made relatively safe, not only to the users, but also to their neighbors and to the animals in their care.

User error or ignorance is the underlying cause for most animal poisonings, and effective education together with the requirement for good common sense is the prime method of reversing the increasing incidence of animal toxicities. Chemicals are weapons--they must be recognized as such, and not just pointed out and fired!

Summary

Animals are constantly exposed to a wide variety of foreign chemicals, many of which are potentially toxic and some which result in clinical poisonings. Pesticides are applied on or around animals for the control of insects and rodents. These chemicals may be placed in areas without regard for accessibility to household pets and domestic livestock. Insecticides, herbicides, and fungicides are routinely and haphazardly applied to animal and environmental surfaces alike with apparent disregard for differences in absorptive capability. Fortunately, the newer herbicides and fungicides are relatively non-hazardous. Drugs are considered to have therapeutic effects; but the lay and professional person's disregard for species differences and variations in recommended dosages can result in poisonings. Adverse reactions may be misnomers for errors in judgment. Failure to provide satisfactory storage facilities for animal feeds and the improper preservation and handling of feedstuffs allow the growth and development of a variety of mycotoxins. Ignorance on the part of animal owners and livestockmen can result in a number of unusual and sometimes fatal clinical syndromes. The dependence of domestic animals and livestock upon their owners for the total environment makes these animals extremely susceptible to environmental pollutants. Exposure to noxious gases, irritating and hazardous industrial materials and wastes, water contaminants, and casually discarded compounds of man's own use can and frequently do result in animal illnesses and death. Persons responsible for animals may be unaware of the potential hazard or lack good judgment in the use of these chemicals. Forceful and continuing education for the safe and sensible use of all foreign compounds on and around domestic animals and livestock is needed. Persons handling and applying these materials must recognize their responsibility, not only to themselves but also to their neighbors and the animals in their care.

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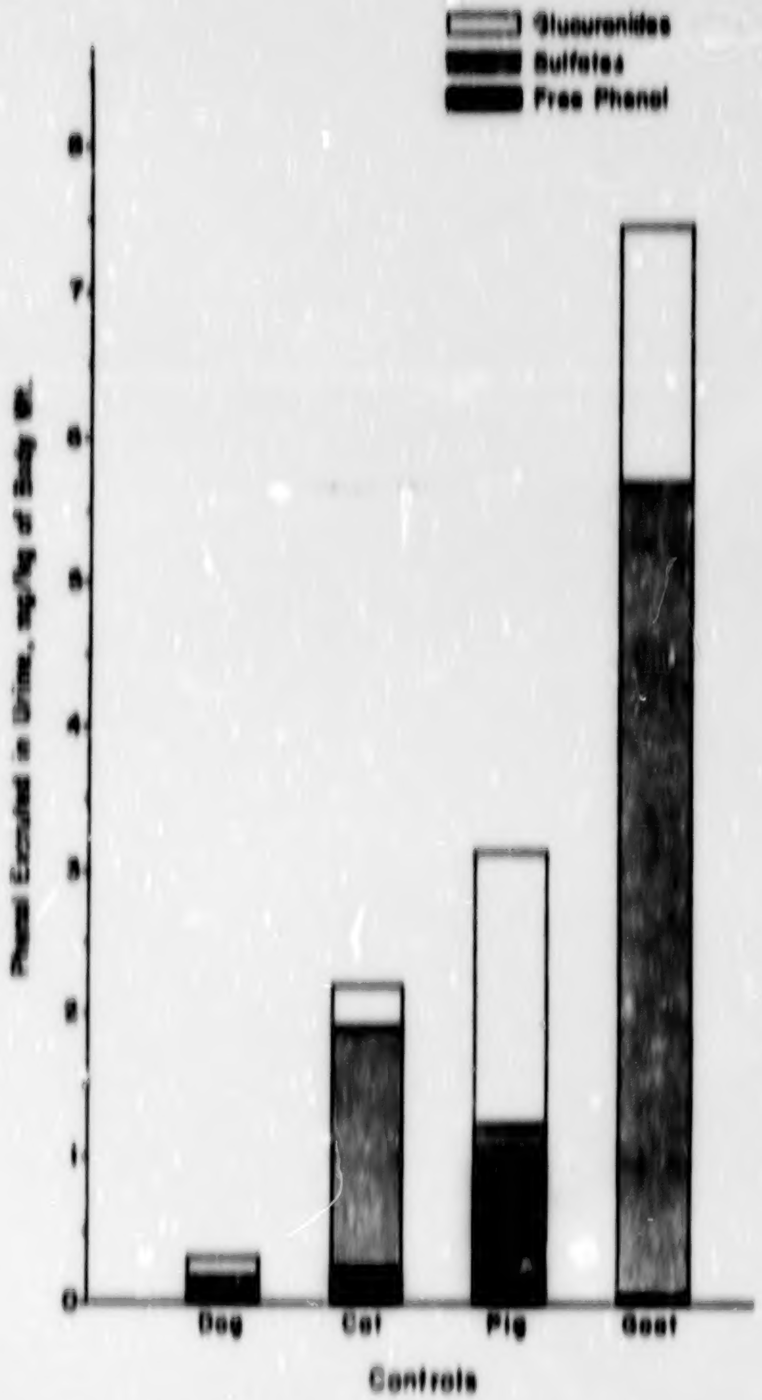

Figure 1 -- Phenolic components of urine from 4 different species of control animals.

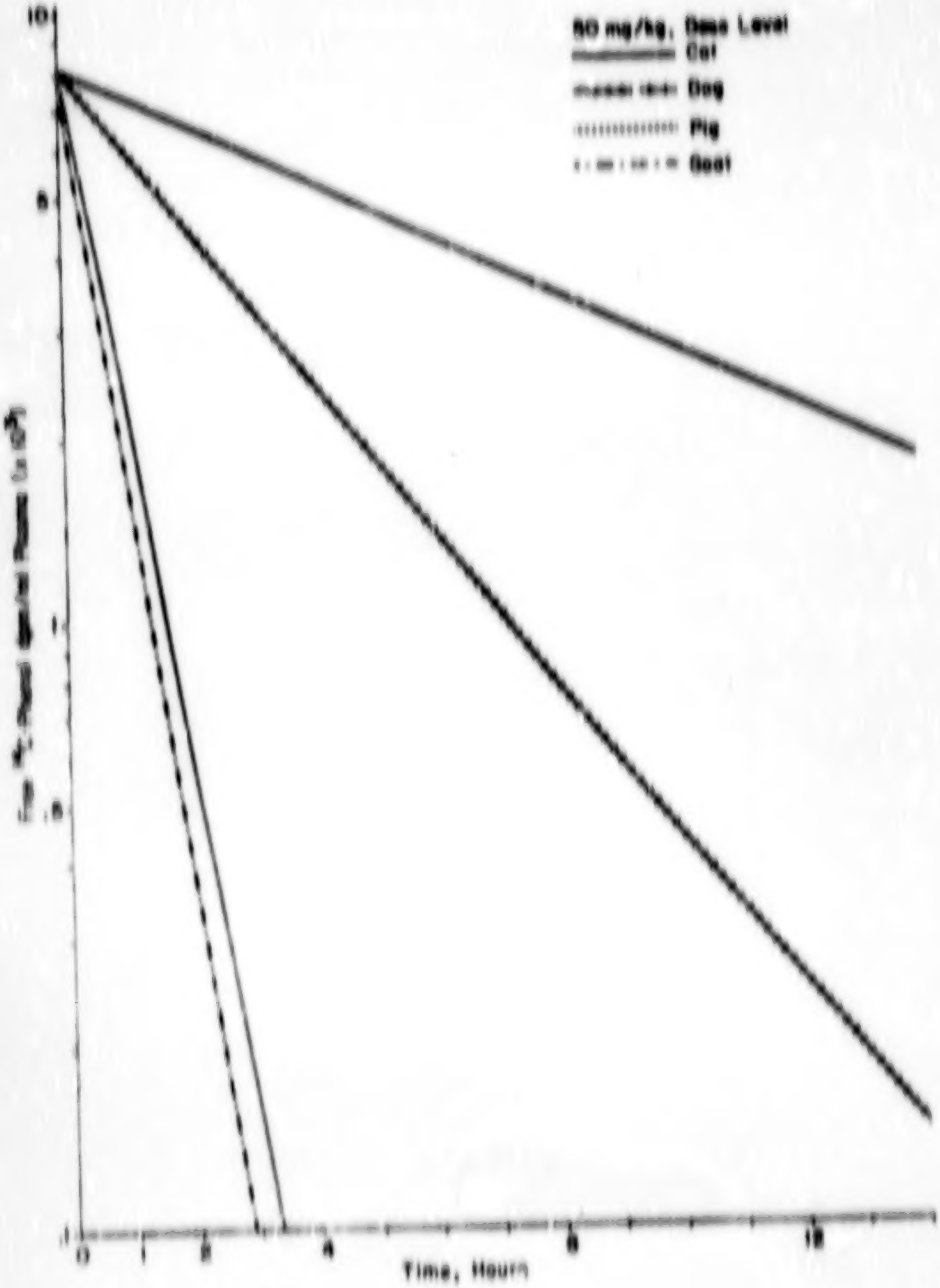

Figure 2 -- Plasma disappearance of identical iv doses of ^{14}C-phenol in 4 animal species.

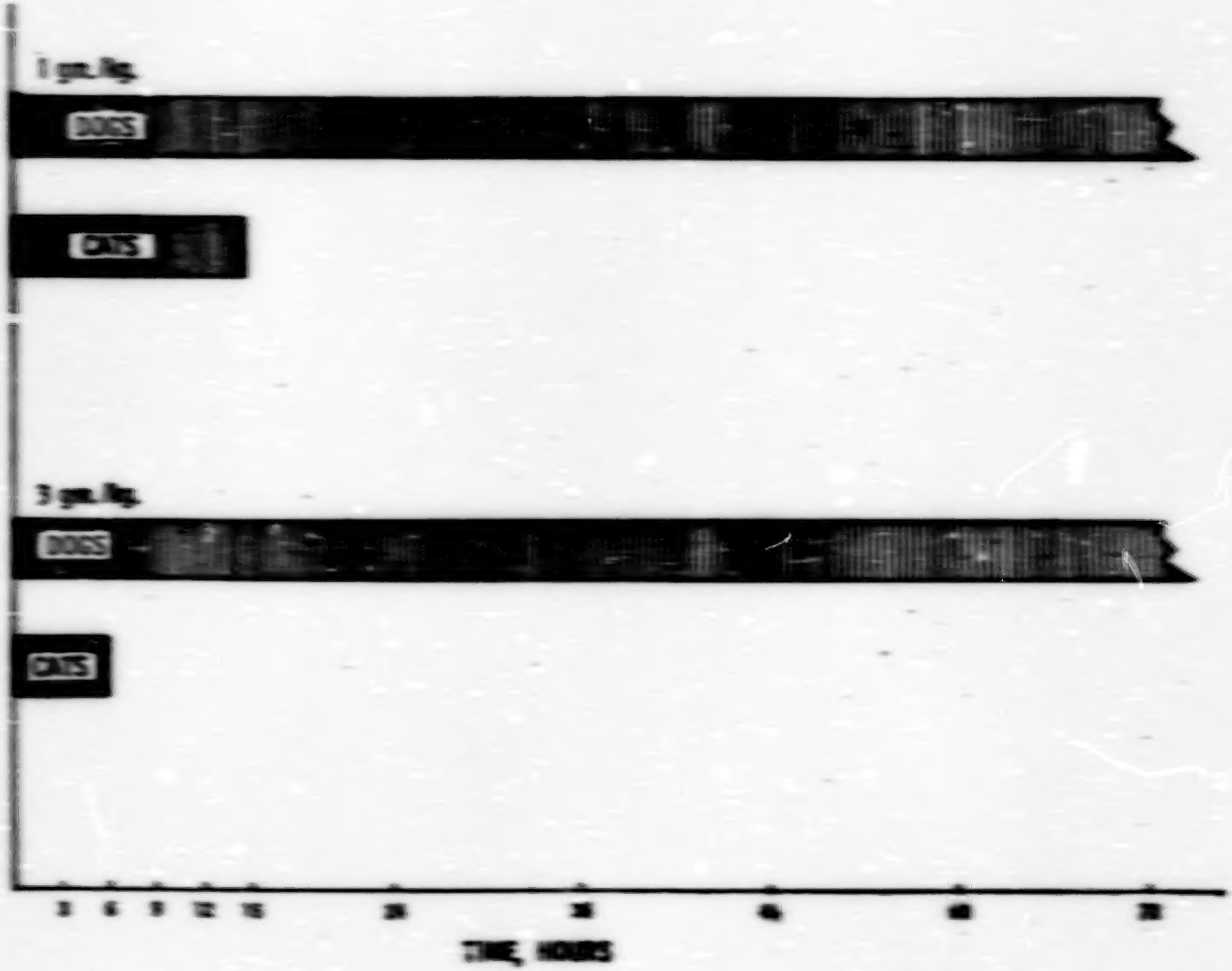

Figure 3 — Mean survival times for dogs and cats given 1 dose of a phenolic compound.

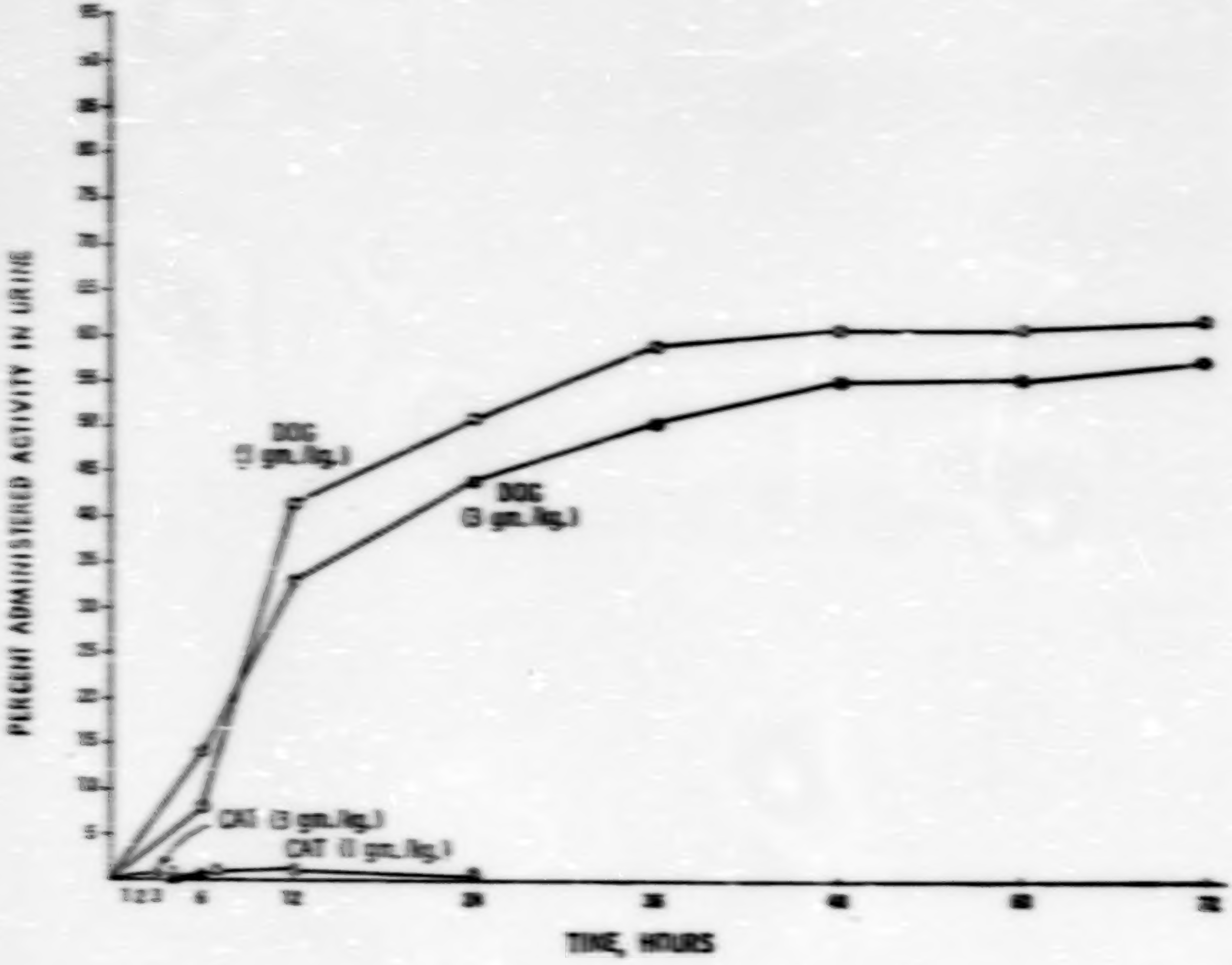

Figure 4 — Urinary excretion of a phenolic compound in dogs and cats receiving doses.

Table 1 -- Mismanagement and the Development of Urea Poisoning

1. Only roughage fed before urea offered
2. No previous urea fed
3. Switch to high urea ration suddenly
4. "Bully cattle" hog feed
5. Cattle unusually hungry, overeat
6. Feed instructions not followed
7. Accidently fed wrong mixture
8. Improper or incomplete feed mixing

Table 2 -- Percentage of Water Samples Analyzed with Various Nitrate Concentrations

Range (ppm NO_3)	Samples (%)
0-10.9	41.9
11-19.9	13.4
20-29.9	7.3
30-39.9	5.0
40-49.9	3.9
50-59.9	7.3
60-69.9	2.2
70 & over	19.0
	100.0

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
Operations Division, Office of Pesticide Programs
CHAMBLEE, GEORGIA 30341

April 11, 1973

TO ALL COURSE PARTICIPANTS:

A copy is enclosed of the Proceedings from the first annual short course on "Environmental Chemicals: Human and Animal Health" which was held at Colorado State University, Fort Collins, Colorado, August 7-11, 1972. Approximately 110 people from 23 states, Canada and Panama attended. We were pleased that this conference provided an opportunity for exchange of thoughts, information and concepts among the various disciplines represented. Suggestions from attendees for course improvement will be used in developing the second annual short course on Environmental Chemicals: Human and Animal Health. This will be held at Colorado State University during the week of July 22, 1973. Specific details are available on request from Dr. Eldon P. Savage.

Sincerely yours,

Anne R Yobs

Anne R. Yobs, M. D.
Chief, Training & Education Branch

Enclosure

Title 40—Protection of Environment

CHAPTER I—ENVIRONMENTAL PROTECTION AGENCY

[FRL 266-1]

PART 171—CERTIFICATION OF PESTICIDE APPLICATORS

On February 22, 1974, notice was published in the Federal Register (39 FR [illegible]) Part 171) proposing standards for the certification of applicators of restricted use pesticides pursuant to section 4 of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), as amended (86 Stat. 973, 997).

Section 4(a)(1) of the Act provides that "the Administrator shall prescribe standards for the certification of applicators of pesticides. Such standards shall provide that to be certified, an individual must be determined to be competent with respect to the use and handling of pesticides, or to the use and handling of the pesticide or class of pesticides covered by such individual's certification." The regulations, as proposed, established categories of commercial applicators and set forth minimum standards that commercial and private applicators must meet before they are certified to use pesticides classified under section 3 of the Act for "restricted use."

Written comments on the proposed standards were invited and received from interested parties. All of the comments have been reviewed and are on file with the Agency. Certain of the comments have been incorporated into the revised standards. Some of the revisions involved editorial changes for purposes of clarification. Other changes were made in an effort to balance amended FIFRA's requirements to establish certification standards that will assure the proper use of pesticides with the impact of these requirements upon both pesticide applicators and State agencies responsible for establishing and implementing certification programs. Significant comments, modifications and policy issues are described below.

1. General Comments

The actual general and specific standards (171.4 (b) and (c)) contained in the regulations received little adverse comment and several commentators commended the Agency approach. Some commentators suggested, however, that Congress intended certification standards to relate entirely to human safety, that, therefore, the proposed standards went beyond the intent of Congress, and that some States may not be able to implement the standards by the October 1976, deadline.

While there is no doubt that implementation of strict interpretations of the certification standards may temporarily strain the resources of some States, EPA feels that the [illegible] standards as [illegible] presented are reasonable, do not [illegible] reflect genuine concern for [illegible] the environment, and the [illegible] of proper use. Furthermore, EPA feels that the Act sufficiently broad and flexible to allow for implementation in all States by October 1976. Finally, in the Agency's opinion, [illegible]

[illegible]

The proposed standards relate to broad general areas of knowledge that should be covered in an applicator's certification. [illegible] develop reciprocity procedures at their d[illegible]

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eration. Standards for certification of applicators described in these regulations provide sufficient commonality to permit such procedures—provided that differences do not exist between the States involved.

This procedural matter will also be detailed in regulations dealing with approval of State plans.

An industry commentator objected to the use of the term "restricted use pesticides" in the standards and preamble on the basis that this implies that EPA may classify by chemical pesticide rather than on a use-by-use, product-by-product basis. The Agency prefers to use the term "restricted use pesticide," since that term was used in the Act. However, the Agency wants to make it clear that classification will be by pesticide uses—not by the chemical pesticide per se.

A new § 171.4(e) has been added to read as follows:

(e) The above standards do not apply to the following persons for purposes of these regulations:

(1) Persons conducting laboratory type research involving restricted use pesticides; and

(2) Doctors of Medicine and Doctors of Veterinary Medicine applying pesticides as drugs or medication during the course of their normal practice.

Persons conducting laboratory type research are not considered to be applying pesticides for use to which the restricted classification applies (see § 3(d)(1) of amended FIFRA) and they are not therefore using pesticides in the sense contemplated by the Act. Laboratory research workers, by the very nature of their occupation, can safely be presumed to be familiar with the safety requirements of restricted use pesticides and such research does not present an appreciable hazard to the environment. Further, such uses of restricted use pesticides by such researchers would be in connection with tests designed to determine the potential of new chemicals as pesticides rather than for pest control purposes, per se. Accordingly, and in the interest of facilitating exploratory research, EPA feels that such persons do not have to be certified to engage in laboratory type research involving restricted use pesticides.

Field researchers who do require certification are discussed in section-by-section comments of § 171.3(b)(10).

Concern about the status of Veterinarians was expressed in comments by the American Veterinary Medical Association which recommended that provisions of the Act relating to certification would not apply to Doctors of Veterinary Medicine applying pesticides to animals during the course of veterinary medical practice, provided that such Veterinarian is not regularly engaged in the business of applying pesticides for hire (amounting to a principal or regular occupation), or does not publicly hold himself out to be a pesticide applicator.

The recommendation seems reasonable since both Doctors of Medicine and Doctors of Veterinary Medicine are qualified and regulated under other laws. In addition, the products involved (pesticides used as drugs and medications) are regulated under the Federal Food, Drug, and Cosmetic Act. Thus, there is no need to require certification of persons using or prescribing these uses of pesticide products.

Veterinarians requiring certification as pesticide applicators are discussed in section-by-section comments of § 171.3(b)(11)(ii).

B. SPECIFIC COMMENTS ABOUT THE REGULATIONS BY SECTION[1]

§ 171.2(a). It was pointed out that use of the word "unintended" in the definition of "accident" is inaccurate since some undesirable events may well be deliberate. Therefore the words "and unintentional" have been omitted in the final standards, while the word "unexpected" has been added.

§ 171.2(c). It was pointed out that omission of "animal product" eliminates such legitimate agricultural commodities as eggs, milk and honey. Thus the words "or animal product" have been added to the definition.

§ 171.2(e). Many commentators noted that use of the word "practical" in defining the word "competent" implies requiring a much higher level of knowledge than the dictionary definition of "competent." The words "properly qualified" have been substituted for the word "practical" where used in the standards.

§ 171.2(h). EPA's proposed definition of "farmer" elicited more public comments than did any other provision of the proposed standards. The effect of the proposed definition would have been to limit the private applicator category to persons producing agricultural commodities worth at least $1000 per year.

There were objections from farmers and farm organizations, farm groups and State regulatory and extension officials. A major objection was that the proposed definition would discriminate against low income and retired farmers, and especially home gardeners. As proposed, such persons would have to qualify as commercial applicators or they would not be able to use restricted use pesticides. Many commentators objected to inclusion of the term "farmer" in the regulations at all since it is not mentioned in the Act's definition of "private applicator."

State regulatory officials also objected that it would be administratively difficult to implement the proposed definition which involved the requirement of production of agricultural commodities having a gross value of at least $1000 per year or which would be expected to have that or greater value at the time of their sale or use.

After considering the matter, EPA has concluded that the definition need not be retained and that there are other ways to ensure that an individual's access to restricted use pesticides is related to his competence to handle such products.

The farmer definition (and $1000 requirement) had been included in the original proposal to assist State agencies in defining the private applicator and to assist in keeping restricted use products from being easily available to persons whose knowledge and experience in using pesticides is limited, e.g., hobby gardeners. Farmers, and others similarly engaged in agricultural production, usually have long experience and familiarity with pesticides and are, thus, more likely to know how to use a restricted use product properly and safely.

Although it is true that the Act itself does not specifically mention the term "farmer" when defining the term "private applicator," this definition, which refers to "producing agricultural commodities," is clear indication that the private applicator classification was meant to be reserved primarily for farmers and other similar persons. In addition, there were frequent references to farmers in the Legislative History of the Act which is considered in formulating regulations.

Therefore, while eliminating the term "farmer" as a separate definition, along with the dollar value requirement, the concern of limiting the availability of certain highly toxic products to those who regularly employ them and are thus familiar with their potentially dangerous properties [illegible] this concept is continued in the revised definition of the term "agricultural commodity." In addition, other provisions of FIFRA, as amended, will be employed to ensure that pesticide products available to individuals such as home gardeners are products that can be used properly and safely by the general public. Such provisions include pesticide registration and classification and pesticide packaging and labeling.

§ 171.3(a). Several State regulatory officials expressed concern that the phrase "State systems of applicator certification shall adopt these categories" implied that all States would be required to establish all categories whether or not they are needed in a particular State. This section has been clarified by adding the words "as needed."

§ 171.3(b)(11)(ii). In recognition of the fact that some Veterinarians engage in pest control activities beyond their normal veterinary medical practice, a new paragraph was added as follows:

Doctors of Veterinary Medicine engaged in the business of applying pesticides for hire, publicly holding themselves out as pesticide applicators, or engaged in large scale use of pesticides are included in this category.

§ 171.3(b)(10). The proposed section was expanded to include persons conducting field research with pesticides, and in doing so, use or supervise the use of restricted use pesticides. The omission of such persons in the proposed standards apparently implied that they did not need to be certified.

Exemption of field researchers using or supervising the use of restricted use pes-

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[1] Section numbers beginning each sec paragraph refer to the original section numbers in the proposed rule unless preceded with the term "new."

[illegible]

manufacturers, pest control operators, and farm organizations, mainly on the grounds that this provision would have the effect of setting up a "use by prescription" classification, a concept specifically rejected by the Congress.

EPA did not—and still does not—intend to establish any such classification.

It is quite possible, however, that the re-registration and classification process mandated by amended FIFRA will indicate that certain pesticide products/uses require special expertise on the part of applicators if they are to be used without causing unreasonable adverse effects

[illegible]

Federal standards since this is the State prerogative. Therefore, proposed section (c) is deleted and [illegible] guidance from the Legislative History, a sentence to accommodate this situation is inserted in §§ 171.4(c) and 171.5(c).

A new § 171.4(e) has been added to exclude persons conducting laboratory type research involving restricted use pesticides and Doctors of Medicine and Doctors of Veterinary Medicine applying pesticides as drugs or medication during the course of their normal practice (discussed in detail on page 7).

§ 171.5 (a) & (b). Many pest control

[illegible]

thought of discriminating against such people by denying a competent person certification. Consequently, a provision has been included in the administration procedures (§ 171.6(a)(1)) to permit States to develop procedures for assessing the competence of persons who, at the time of examination, cannot read. Such procedures must be incorporated in the relevant State plan which, in turn, must be approved by EPA.

The Act does not require that applicator certification be granted for across-the-board access to restricted use products. Section 4 provides that "an individ-

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ual must be determined to be competent with respect to the use and handling of pesticides, or to the use and handling of the pesticide or class of pesticides covered by such individual's certification. Thus, FIFRA as amended provides that a private applicator can be certified to use just one or a particular group of pesticides needed in his particular agricultural activities. A person, particularly one who cannot read, could learn by oral communication and [illegible] all that he needs to know about a small number of pesticides, but not enough to qualify him to use all restricted use pesticides. Any waiver of the reading requirement, therefore, will have to be on a case-by-case basis based on a plan developed by the State plan and approved by the Administrator. In any case, where a private applicator cannot read a label and labeling information, he must demonstrate (as is the case for all private applicators) his understanding of the requirements of § 171.5(a). Additionally, certification will focus on each individual restricted use pesticide which he intends to use in his agricultural operations and he will be authorized to use only those pesticides for which he has demonstrated competency. With respect to § 171.5(a)(2), where the ability to read a label is required, the non-reader would be tested on his understanding of the label and labeling information and would [illegible] pattern of advice and guidance on the safe and proper use of pesticides. A State must also develop as part of its plan, specific procedures for approving certification for such an applicator who plans to use a new pesticide in his operations.

In order that EPA may provide guidance to the States in the near future regarding acceptable procedures to use in assessing the competency of those who cannot read a label, EPA is soliciting proposals for appropriate procedures to be used in assessing the competence of these private applicators. Acceptable procedures will be published as part of the guidelines to certification procedures.

Effective date: October 9, 1974.

JOHN QUARLES,
Acting Administrator.

OCTOBER 1, 1974.

A new part 171 is added, effective October 9, 1974, to read as follows:

Sec.
171.1 General.
171.2 Definitions.
171.3 Categorization of commercial applicators of pesticides.
171.4 Standards for certification of commercial applicators.
171.5 Standards for certification of private applicators.
171.6 Standards for supervision of noncertified applicators by certified private and commercial applicators.

AUTHORITY: Section 4, Federal Insecticide, Fungicide, and Rodenticide Act, as amended, 86 Stat. 983, 997.

§ 171.1 General.

This section deals with the certification of applicators of restricted use pesticides.

§ 171.2 Definitions.

Terms used in this subpart shall have the meanings set forth for such terms in the Act. In addition, as used in this subpart, the following terms shall have the meanings stated below:

(a) The term "accident" means an unexpected, undesirable event, caused by the use or presence of a pesticide, that adversely affects man or the environment.

(b) The term "Act" means the Federal Insecticide, Fungicide, and Rodenticide Act, as amended (86 Stat. 973), and other applicable supplementary statutes and amendments thereof.

(c) The term "Administrator" means the Administrator of the Environmental Protection Agency, or any officer or employee of the Agency to whom authority has heretofore been delegated, or to whom authority may hereafter be delegated, to act in his stead.

(d) The term "Agency," unless otherwise specified, means the United States Environmental Protection Agency.

(e) The term "agricultural commodity" means any plant, or part thereof, or animal, or animal product, produced by a person (including farmers, ranchers, vineyardists, plant propagators, Christmas tree growers, aquaculturists, floriculturists, orchardists, foresters, or other comparable persons) primarily for sale, consumption, propagation, or other use by man or animals.

(f) The term "calibration of equipment" means measurement of dispersal or output of application equipment and adjustment of such equipment to control the rate of dispersal, and droplet or particle size of a pesticide dispersed by the equipment.

(g) The term "certification" means the recognition by a certifying agency that a person is competent and thus authorized to use or supervise the use of restricted use pesticides.

(h) The term "certified applicator" means any individual who is certified to use or supervise the use of any restricted use pesticide covered by his certification.

(i) The term "commercial applicator" means a certified applicator (whether or not he is a private applicator with respect to some uses) who uses or supervises the use of any pesticide which is classified for restricted use for any purpose or on any property other than as provided by the definition of "private applicator."

(j) The term "compatibility" means that property of a pesticide which permits its use with other chemicals without undesirable results being caused by the combination.

(k) The term "competent" means properly qualified to perform functions associated with pesticide application, the degree of capability required being directly related to the nature of the activity and the associated responsibility.

(l) The term "common exposure route" means a likely way (oral, dermal, respiratory) by which a pesticide may reach and/or enter an organism.

(m) The term "environment" means water, air, land, and all plants and man and other animals living therein, and the interrelationships which exist among these.

(n) The term "forest" means a concentration of trees and related vegetation in non-urban areas sparsely inhabited by and infrequently used by humans; characterized by natural terrain and drainage patterns.

(o) The term "hazard" means a probability that a given pesticide will have an adverse effect on man or the environment in a given situation, the relative likelihood of danger or ill effect being dependent on a number of interrelated factors present at any given time.

(p) The term "host" means any plant or animal on or in which another lives for nourishment, development, or protection.

(q) The term "non-target organism" means a plant or animal other than the one against which the pesticide is applied.

(r) The term "ornamental" means trees, shrubs, and other plantings in and around habitations generally, but not necessarily located in urban and suburban areas, including residences, parks, streets, retail outlets, industrial and institutional buildings.

(s) The term "practical knowledge" means the possession of pertinent facts and comprehension together with the ability to use them in dealing with specific problems and situations.

(t) The term "private applicator" means a certified applicator who uses or supervises the use of any pesticide which is classified for restricted use for purposes of producing any agricultural commodity on property owned or rented by him or his employer or (if applied without compensation other than trading of personal services between producers of agricultural commodities) on the property of another person.

(u) The term "protective equipment" means clothing or any other materials or devices that shield against unintended exposure to pesticides.

(v) The term "regulated pest" means a specific organism considered by a State or Federal agency to be a pest requiring regulatory restrictions, regulations, or control procedures in order to protect the host, man and/or his environment.

(w) The term "restricted use pesticide" means a pesticide that is classified for restricted use under the provisions of section 3(d)(1)(C) of the Act.

(x) The term "standard" means the measure of knowledge and ability which must be demonstrated as a requirement for certification.

(y) The term "State" means a State, the District of Columbia, the Commonwealth of Puerto Rico, the Virgin Islands, Guam, the Trust Territory of the Pacific Islands, and American Samoa.

(z) The term "susceptibility" means the degree to which an organism is affected by a pesticide at a particular level of exposure.

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(aa) The term "toxicity" means the property of a pesticide to cause any adverse physiological effects.

(bb) The term "under the direct supervision of" means the act or process whereby the application of a pesticide is made by a competent person acting under the instructions and control of a certified applicator who is responsible for the actions of that person and who is available if and when needed, even though such certified applicator is not physically present at the time and place the pesticide is applied.

§ 171.3 Categorization of commercial applicators of pesticides.

(a) *Procedure.* Categories of applicators (other than private) using or supervising the use of restricted use pesticides are identified below. State systems of applicator identification shall adopt these categories as needed along with such appropriate subcategories as are necessary to meet the particular requirements of the State.

(b) *Categories*—(1) *Agricultural pest control*—(i) *Plant.* This category includes commercial applicators using or supervising the use of restricted use pesticides in production of agricultural crops, including without limiting the foregoing, tobacco, peanuts, cotton, feed grains, soybeans and forage; vegetables, small fruits, tree fruits and nuts; as well as on grasslands and non-crop agricultural lands.

(ii) *Animal.* This category includes commercial applicators using or supervising the use of restricted use pesticides on animals, including without limiting the foregoing beef cattle, dairy cattle, swine, sheep, horses, goats, poultry, and livestock, and to places on or in which animals are confined.

Doctors of Veterinary Medicine engaged in the business of applying pesticides for hire, publicly holding themselves out as pesticide applicators, or engaged in large-scale use of pesticides are included in this category.

(2) *Forest pest control.* This category includes commercial applicators using or supervising the use of restricted use pesticides in forests, forest nurseries, and forest seed producing areas.

(3) *Ornamental and turf pest control.* This category includes commercial applicators using or supervising the use of restricted use pesticides to control pests in the maintenance and production of ornamental trees, shrubs, flowers, and turf.

(4) *Seed treatment.* This category includes commercial applicators using or supervising the use of restricted use pesticides on seeds.

(5) *Aquatic pest control.* This category includes commercial applicators using or supervising the use of any restricted use pesticide purposefully applied to standing or running water, excluding applicators engaged in public health related activities included in category 8 below.

(6) *Right-of-way pest control.* This category includes commercial applicators using or supervising the use of restricted use pesticides in the maintenance of public roads, electric powerlines, pipelines, railway rights-of-way or other similar areas.

(7) *Industrial, institutional, structural and health related pest control.* This category includes commercial applicators using or supervising the use of restricted use pesticides in, on, or around food handling establishments, human dwellings, institutions, such as schools and hospitals, industrial establishments, including warehouses and grain elevators, and any other structures and adjacent areas, public or private; and for the protection of stored, processed, or manufactured products.

(8) *Public health pest control.* This category includes State, Federal or other governmental employees using or supervising the use of restricted use pesticides in public health programs for the management and control of pests having medical and public health importance.

(9) *Regulatory pest control.* This category includes State, Federal or other governmental employees who use or supervise the use of restricted use pesticides in the control of regulated pests.

(10) *Demonstration and research pest control.* This category includes: (i) Individuals who demonstrate to the public the proper use and techniques of application of restricted use pesticides or supervise such demonstration; and (ii) persons conducting field research with pesticides, and in doing so, use or supervise the use of restricted use pesticides. Included in the first group are such persons as extension specialists and county agents, commercial representatives demonstrating pesticide products, and those individuals demonstrating methods used in public programs. The second group includes State, Federal, commercial and other persons conducting field research on or utilizing restricted use pesticides.

(c) *Other categories and subcategories.* Any State submitting a plan pursuant to this section for the certification of applicators, as provided for below, may designate such subcategories within the above 10 categories as it deems necessary. In addition, a State may delete a category not needed or may request the Administrator's approval of additional major categories.

§ 171.4 Standards for certification of commercial applicators.

(a) *Determination of competency.* Competence in the use and handling of pesticides shall be determined on the basis of written examinations and, as appropriate, performance testing, based upon standards set forth below and which are approved by the Administrator. Such examination and testing shall include the general standards applicable to all categories (section 171.4(b)) and the additional standards specifically identified for each category or subcategory (if any) in which an applicator is to be classified (section 171.4(c)). State standards must conform and be at least equal to those prescribed herein. In developing the details of standards at the State level and in structuring examinations, it is important to recognize and reflect the extent of competency appropriate and necessary to a particular category.

(b) *General standards for all categories of certified commercial applicators.* (1) All commercial applicators shall demonstrate practical knowledge of the principles and practices of pest control and safe use of pesticides. Testing shall be based on examples of problems and situations appropriate to the particular category or subcategory of the applicator's certification and the following areas of competency:

(i) *Label & labeling comprehension.* (a) The general format and terminology of pesticide labels and labeling;

(b) The understanding of instructions, warnings, terms, symbols, and other information commonly appearing on pesticide labels;

(c) Classification of the product, general or restricted; and

(d) Necessity for use consistent with the label.

(ii) *Safety.* Factors including: (a) Pesticide toxicity and hazard to man and common exposure routes;

(b) Common types and causes of pesticide accidents;

(c) Precautions necessary to guard against injury to applicators and other individuals in or near treated areas;

(d) Need for and use of protective clothing and equipment;

(e) Symptoms of pesticide poisoning;

(f) First aid and other procedures to be followed in case of a pesticide accident; and

(g) Proper identification, storage, transport, handling, mixing procedures and disposal methods for pesticides and used pesticide containers, including precautions to be taken to prevent children from having access to pesticides and pesticide containers.

(iii) *Environment.* The potential environmental consequences of the use and misuse of pesticides as may be influenced by such factors as: (a) Weather and other climatic conditions;

(b) Types of terrain, soil or other substrate;

(c) Presence of fish, wildlife and other non-target organisms; and

(d) Drainage patterns.

(iv) *Pests.* Factors such as: (a) Common features of pest organisms and characteristics of damage needed for pest recognition;

(b) Recognition of relevant pests; and

(c) Pest development and biology as it may be relevant to problem identification and control.

(v) *Pesticides.* Factors such as: (a) Types of pesticides;

(b) Types of formulations;

(c) Compatibility, synergism, persistence and animal and plant toxicity of the formulations;

(d) Hazards and residues associated with use;

(e) Factors which influence effectiveness or lead to such problems as resistance to pesticides; and

(f) Dilution procedures.

(vi) *Equipment.* Factors including:

(a) Types of equipment and advantages and limitations of each type; and

(b) Uses, maintenance and calibration.

(viii) *Application techniques.* Factors including: (a) Methods of procedure used to apply various formulations of pesticides, solutions, and gases, together with a knowledge of which technique of application to use in a given situation;

(b) Relationship of discharge and placement of pesticides to proper use, unnecessary use, and misuse; and

(c) Prevention of drift and pesticide loss into the environment.

(ix) *Laws and regulations.* Applicable State and Federal laws and regulations.

(c) *Specific standards of competency for each category of commercial applicators.* [illegible]

(1) *Agricultural pest control*—(i) *Plant.* [illegible] areas.

(ii) *Animal.* Applicators applying pesticides directly to animals shall demonstrate practical knowledge of such animals and their associated pests. [illegible] applicator must know the relative hazards associated with such factors as formulation, application techniques, age of animals, stress and extent of treatment.

(2) *Forest pest control.* Applicators shall demonstrate practical knowledge of types of forests, forest nurseries, and seed production in their State and the pests involved. [illegible]

(3) *Ornamental and turf pest control.* [illegible]

(4) *Seed treatment.* [illegible]

(5) *Aquatic pest control.* [illegible]

(6) *Right-of-way pest control.* Applicators shall demonstrate practical knowledge of a wide variety of environments, since rights-of-way can traverse many different terrains, including waterways. They shall demonstrate practical knowledge of problems on runoff, drift, and excessive foliage destruction and ability to recognize target organisms. They shall also demonstrate practical knowledge of the nature of herbicides and the need for containment of these pesticides within the right-of-way area, and the impact of their application activities in the adjacent areas and communities.

(7) *Industrial, institutional, structural and health related pest control.* Applicators must demonstrate a practical knowledge of a wide variety of pests, including their life cycles, types of formulations appropriate for their control, and methods of application that avoid contamination of food, damage and contamination of habitat, and exposure of people and pets. [illegible]

(8) *Public health pest control.* [illegible]

(9) *Regulatory pest control.* [illegible]

(10) *Demonstration and research pest control.* [illegible] reflecting a broad spectrum of pesticide uses. Many different pest problem situations will be encountered in the course

of activities associated with demonstration, and practical knowledge of problems, pests and population levels occurring in each demonstration situation is required. Further, they should demonstrate an understanding of pesticide-organism interactions and the importance of integrating pesticide use with other control methods. In general, it would be expected that applicators doing demonstration pest control work possess a practical knowledge of all of the standards detailed in section 171.4(b). In addition, they shall meet the specific standards required for categories (1) through (7) of this section as may be applicable to their particular activity.

Persons conducting field research or method improvement work with restricted use pesticides should be expected to know the general standards detailed in 171.4(b). In addition, they shall be expected to know the specific standards required for categories (1) through (7) of this section applicable to their particular activity or alternatively to meet the more inclusive requirements listed under "Demonstration."

(11) *Special standards.* This space reserved for possible issuance of Special Standards.

(d) The above standards do not apply to the following persons for purposes of their certification: (1) Persons conducting laboratory type research involving restricted use pesticides; and (2) Doctors of Medicine and Doctors of Veterinary Medicine applying pesticides as drugs or medication during the course of their normal practice.

§ 171.5 Standards for certification of private applicators.

(a) Competence in the use and handling of pesticides by a private applicator will be determined by procedures set forth below. State standards must conform and be at least equal to those prescribed herein. As a minimum requirement for certification, a private applicator must show that he possesses a practical knowledge of the pest problems and pest control practices associated with his agricultural operations; proper storage, use, handling, and disposal of the pesticides and containers; and his related legal responsibility. This practical knowledge includes ability to:

(1) Recognize common pests to be controlled and damage caused by them.

(2) Read and understand the label and labeling information—including the common name of pesticides he applies; pest(s) to be controlled, timing and methods of application; safety precautions; any pre-harvest or re-entry restrictions; and any specific disposal procedures.

(3) Apply pesticides in accordance with label instructions and warnings, including the ability to prepare the proper concentration of pesticide to be used under particular circumstances taking into account such factors as area to be covered, speed at which application equipment will be driven, and the quantity dispersed in a given period of operation.

(4) Recognize local environmental situations that must be considered during application to avoid contamination.

(5) Recognize poisoning symptoms and procedures to follow in case of a pesticide accident.

(b) Such competence of each private applicator shall be verified by the appropriate State agency through the administration of a private applicator certification system which ensures that the private applicator is competent, based upon the standards set forth above, to use the restricted use pesticides under limitations of applicable State and Federal laws and regulations. A certification system shall employ a written or oral testing procedure, or such other equivalent system as may be approved as part of a State plan.

(c) In any case where a person, at the time of testing for certification, is unable to read a label, the responsible State agency may employ a testing procedure (previously approved by the Administrator) which can adequately assess the competence of such person with regard to all of the above standards. Certification must be related and limited to the use and handling of each individual pesticide for which he desires certification at any time. Therefore, the applicator will be authorized to use only the pesticide(s) for which he has demonstrated competence. A specific procedure is required for section 171.5(a)(2) relative to label comprehension, with testing designed to assure his knowledge of the following:

(1) Understanding of the label and labeling information, including those items indicated in that subsection.

(2) Sources of advice and guidance necessary for the safe and proper use of each pesticide related to his certification.

§ 171.6 Standards for supervision of non-certified applicators by certified private and commercial applicators.

Certified applicators whose activities indicate a supervisory role must demonstrate a practical knowledge of Federal and State supervisory requirements, including labeling, regarding the application of restricted use pesticides by noncertified applicators.

The availability of the certified applicator must be directly related to the hazard of the situation. In many situations, where the certified applicator is not required to be physically present, "direct supervision" shall include verifiable instruction to the competent person, as follows: (1) detailed guidance for applying the pesticide properly, and (2) provisions for contacting the certified applicator in the event he is needed. In other situations, and as required by the label, the actual physical presence of a certified applicator may be required when application is made by a noncertified applicator.

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